THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Metabolic Effects of Whole Grains: Emphasis on Glycemic control, Appetite, and Body Weight

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Abstract

Background: Overweight and obesity are among the most pressing health challenges of today, and leading risk factors for premature death. Consequently, effective lifestyle strategies for prevention and treatment are urgently needed. High whole grain intake has consistently been associated with lower BMI, body fat mass, and reduced risk of type 2 diabetes, heart disease, and colorectal cancer. However, few intervention studies designed to study the effect of different whole grains on body weight and metabolic outcomes have been conducted. Wholegrain rye has shown promise in improving metabolic regulation, appetite control, and weight-loss, potentially mediated by changes in gut microbiota and derived metabolites.

Aim: The overall aim of this thesis was to evaluate whether replacing refined grains with whole grains in habitual diets improves metabolic markers, appetite control, and body weight in individuals with elevated cardiometabolic disease risk. Additionally, the aim was to explore the role of metabolic status, gut microbiota, and appetite in mediating diet-induced weight-loss, with focus on wholegrain rye.

Results: Consuming largely intact versus finely milled whole grains resulted in improved glycemic control in individuals with type 2 diabetes. Wholegrain rye foods reduced postprandial glycemia in non-diabetics compared to refined wheat, while incretin responses were similar between diets. Contrary to previous findings, wholegrain rye as part of a 12-week hypocaloric diet did not lead to greater weight-loss but did confirm reductions in CRP, changes in gut microbiota and short-chain fatty acids, potentially promoting cardiometabolic health. Overall, self-reported appetite responses showed no differences between diets and no associations with weight-loss. Individuals with elevated HOMA-IR, CRP, and plasma acetate experienced modest weight and fat mass reductions, particularly following the wheat-based diet.

Conclusion: Despite similar weight and fat mass loss between wholegrain rye and refined wheat diets, associations with metabolic markers suggest that individuals with elevated inflammation and insulin resistance may benefit more from rye foods, while neither appetite-regulating properties of rye nor baseline gut microbiota were associated with weight-loss. Additionally, the rye versus wheat foods altered gut microbiota, increased short-chain fatty acids, and reduced inflammatory markers which may be beneficial for long-term health.

Keywords: Overweight and Obesity; Wholegrain rye; Satiety and appetite regulation; Wholegrain particle size; Glycemic response; Gut hormones; Inflammatory markers; Gut microbiota and short-chain fatty acids; Determinants of weight-loss

LIST OF PUBLICATIONS

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Sebastian Åberg**, Jim Mann, Silke Neumann, Alastair B. Ross, and Andrew N. Reynolds. Whole-Grain Processing and Glycemic Control in Type 2 Diabetes: A Randomized Crossover Trial. Diabetes Care 2020;43(8):1717–1723.

II. **Sebastian Åberg**, Marie Palmnäs-Bédard, Therese Karlsson, Thérése Hjorth, Kia Nøhr Iversen and Rikard Landberg. Evaluation of Subjective Appetite Assessment under Free-Living vs. Controlled Conditions: A Randomized Crossover Trial Comparing Whole-Grain Rye and Refined Wheat Diets (VASA-Home). Nutrients 2023, 15(11), 2456.

III. **Sebastian Åberg**, Dominic L. Webb, Elise Nordin, Per Hellström, Rikard Landberg. Day-long Postprandial Effects of Wholegrain Rye Cereals within a Complex Diet: Incretin Hormones, Ghrelin, Blood Glucose, and Inflammation Markers. (Pending revision).

IV. **Sebastian Åberg**, Elise Nordin, Kia Nøhr Iversen, Ingvar Bosaeus, Per Hellström, Karsten Kristiansen, Rikard Landberg. Effects of Hypocaloric Wholegrain Rye vs Refined Wheat Diets on Weight Loss, Cardiometabolic Risk Factors and Gut Microbiota: A 12-Week Randomized Controlled Trial. (Manuscript).

Published papers not included in the thesis:

- Reynolds AN, **Åberg S**, Diep Pham HT, Mann JI, Broadbent JM. Does processing of wholegrain foods affect salivary pH or dental plaque accumulation ? A randomised crossover trial among adults with type 2 diabetes. NZ Dental Journal. 2020;116(October):115–9.

- Palmnäs-Bedard MSA, Costabile G, Vetrani C, **Åberg S**, Hjalmarsson Y, Dicksved J, et al. The human gut microbiota and glucose metabolism: a scoping review of key bacteria and the potential role of SCFAs. Am J Clin Nutr. 2022;116(4):862–74.

- Reynolds AN, Diep Pham HT, **Åberg S**, Neumann S, Mann J. The effects of wholegrain processing on appetite: randomised crossover trial in adults with type 2 diabetes. Food Funct. 2023;7240–6.

Contribution report

Paper I. Sebastian Åberg (SÅ) conducted the study and collected the data. SÅ was responsible for storage and preparation of blood samples and analysis of grain particle size characteristics. SÅ was also responsible for data management of the trial and contributed to interpretation of the data, revision of manuscript drafts, and approval of the final manuscript.

Paper II and III. SÅ conducted the study and collected the data. SÅ contributed in conceptualization and design of the study and in writing the study protocol and ethical application. SÅ conducted analysis of gut hormones through immunoassays and curated data together with an expert. SÅ was responsible for data processing, statistical analysis and writing the manuscripts. SÅ coordinated editing of the manuscripts and the publication process.

Paper IV. SÅ conducted the study and collected the data. SÅ coordinated the sample analysis and was responsible for data management as well as statistical analysis and interpretation of the data. SÅ wrote the first draft of the manuscript and revised the manuscript together with co-authors.

Preface

This dissertation was submitted as a central element in fulfilment of the degree of Doctor of Philosophy at the Department of Life Sciences, Chalmers University of Technology. The work was supported by the governmental research council Formas, grant number [00542. 2014] awarded to Prof. Rikard Landberg. Funding was also provided from Barilla and Lantmännen to conduct the study reported in Paper IV.

The PhD studies were carried out between June 2020 and November 2024 under the supervision of Prof. Rikard Landberg and the co-supervision of Dr. Marie Palmnäs-Bédard, Dr. Elise Nordin, and Dr. Kia Nøhr Iversen with Prof. Ann-Sofie Sandberg as examiner. Most of the work presented in this thesis was carried out at the Division of Food and Nutrition Science at the Department of Life Sciences at Chalmers University of Technology. The first study was conducted at the Department of Medicine, University of Otago, Dunedin with principal investigator Prof. Jim Mann and Dr. Andrew Reynolds as supervisors, supported by grants from Otago Medical Research Foundation and The Riddet Centre of Research Excellence. Analysis of gut peptides was conducted during a research visit at Uppsala University, Department of Medical Sciences, Gastroenterology and Hepatology at Prof. Per Hellström's laboratory under supervision of Dr. Dominic-Luc Webb.

Sebastian Åberg,
November 2024

Abbreviations

AR	Alkylresorcinols
AUC	Area under the curve
BP	Blood pressure
BMI	Body mass index
CCK	Cholecystokinin
CGM	Continuous glucose monitoring system
Cmax	Peak glucose concentration
CRP	C-reactive protein
CONGA	Continuous Overall Improvement in Net Glycemic Action
CV	Coefficient of Variation
DXA	Dual energy x-ray absorptiometry
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
GlycA	Glycoprotein N-acetylation A
GlycB	Glycoprotein N-acetylation B
HbA1c	Glycated hemoglobin A1c
HDL	High-density lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
LDL	Low density lipoprotein
MAGE	Mean Amplitude of Glycemic Excursion
PYY	Peptide tyrosine tyrosine
SCFAs	Short chain fatty acids
SPC	Supramolecular phospholipid composite peak
VAS	Visual analogue scale

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1 INTRODUCTION

Obesity is a leading cause of morbidity and mortality globally, with almost 10% of deaths being a consequence of obesity [1]. About 40% of annual premature deaths due to high BMI is related to persons with BMI <30, highlighting the impact of overweight and not just severe obesity [2]. Adult obesity has doubled, and adolescent obesity quadrupled in the last 30 years. According to data from the World Health Organization, 2.5 billion adults were overweight which of 890 million were obese in 2022 [3].

The balance between energy intake and energy expenditure determines the development of an individual's body weight and a disrupted balance drives the development of overweight, body mass index (BMI) > 25 kg/m^2 and obesity BMI > 30 kg/m^2 over time [4]. Genetic factors are known to affect the individual predisposition for obesity with rare early-onset monogenic obesity, polygenic obesity risk genes, and not the least genetic environment interactions [5,6]. With that in mind, alterations in the human genome do not explain the rapid increase in obesity in the last 30 years. Instead, the shift toward more obesogenic environments in many parts of the world has probably played a key role. With increased wealth and urbanization, the social interplay, mass-media, advertisements and physical surroundings have changed and influence the physical activity, food choices and eating habits [7]. The environment together with individual factors such as education level, socioeconomic status and medical conditions greatly influences personal behavioral patterns and development of overweight and obesity [6,7]. The drivers of obesogenic development are complex, and it is likely an interplay of factors, where a balanced energy intake is fundamental. Therefore, lifestyle strategies that influence dietary patterns and promote healthy food choices are critically needed.

An important factor in body weight management is appetite regulation, including hunger, satiety and desire to eat [8]. This has recently been illustrated by new drug therapies developed based on endogenous hormones central in appetite regulation [9]. Satiety properties of different foods may influence energy intake and dietary strategies that emphasize foods inducing fullness and reducing hunger have the potential to prevent excessive consumption [10,11]. Wholegrain cereals have shown beneficial effects on subjective appetite control when compared with refined alternatives and may be a key factor in observed associations of whole grain intake and reduced obesity and cardiometabolic disease risk [12]. High whole grain intake has consistently been associated with lower BMI, body fat mass, obesity and reduced incidence of related conditions such as type 2 diabetes, heart disease and colorectal cancer [13].

Cereals are important staples and provide >50% of daily caloric intake and represent the most important food source of dietary fiber and plant-based proteins, globally [14,15]. Hence, dietary interventions with wholegrain cereals high in dietary fibre have the potential to reduce body weight and improve body composition. While epidemiological data consistently show reduced risk of overweight and obesity with high intake of whole grain, there has been few intervention studies and results are inconsistent [16]. Contradictory findings in intervention studies are likely linked to design issues, as many studies reporting body weight changes aren't designed for this purpose, often resulting in insufficient intervention periods to demonstrate weight loss. There is

large heterogeneity across studies and effects on body weight and body composition seem to vary, not only by study design, but also with type of cereal investigated [17]. Different cereals vary in nutritional content, microstructure, dietary fiber and bioactive compounds [18], and their effects on appetite have rarely been studied separately [19].

Wholegrain rye has the highest content of dietary fibre among all cereals and has shown beneficial effects on subjective appetite control when compared with refined alternatives [20–24]. However, these interventions have been conducted in controlled clinical conditions and free-living participants have rarely been studied, nor have individuals with overweight or obesity. This group is known for disrupted appetite regulation [25,26] and increased vulnerability to weight gain, metabolic syndrome, and develop diabetes compared to age-matched groups with lower BMI [27,28].

Circulating appetite-regulating hormones can be measured in the postprandial phase, providing objective measures of appetite responses to meals [8]. Hormones like Ghrelin, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) are secreted in response to food intake and are linked to hunger and satiety [29]. Therefore, changes in these hormones may influence subsequent energy intake and body weight regulation. Few studies have measured these gut hormones in response to wholegrain rye foods and results vary with type of foods assessed and study population characteristics [21,23,24,30].

Furthermore, acute meal studies have shown beneficial effects of wholegrain foods on postprandial glycemia, when compared with refined alternatives [20]. High wholegrain intake could potentially benefit long-term glycemic control and prevent development insulin resistance and type 2 diabetes. Oats and barley, rich in β-glucans have repeatedly been reported to improve measures of glycemia, while acute effects of rye have shown inconclusive results [31]. Observational data suggest that high intake of wholegrain rye may reduce the risk of type 2 diabetes, emphasizing the need for more studies on this topic [32]. Observed differences in postprandial responses may be attributed differences in types and amounts of fibre in different grains [33], but also degree of processing [34,35]. Processing of wholegrains, specifically milling has recently been shown to influence postprandial glycemic response to foods derived from the same grain [34]. Indicating structural difference may have a broader impact on postprandial metabolic response.

There are large individual differences in how people respond to dietary interventions, and it has been shown that inter-individual weight loss variability is substantial following hypocaloric dietary interventions [36,37]. Beyond recognized factors such as age, gender, and diet adherence, also individual thermogenic response, gut microbiota, and gut peptides have been suggested as determinants of weight loss [36]. Individual microbiota composition has been suggested as a determinant of high dietary fibre mediated weight loss [38,39], although most studies show no associations with baseline microbiota spp. or enterotypes and weight or body composition changes [37,40,41]. Increased intake of whole grains may induce changes to the microbiota composition and thereby fermentation products such as short chain fatty acids (SCFA), which are known as essential signaling molecules in appetite regulation. Some studies [42], but not all have been able to establish such alterations of the gut microbiome [41].

Intervention studies evaluating satiating effects of whole grains have rarely focused on individuals with overweight or obesity, nor have interventions evaluating metabolic effects of whole grains. There is a need for well-designed intervention studies to elucidate efficacy of whole grain interventions on body weight and body composition in overweight and obese individuals. To improve the understanding of biochemical processes mediating metabolic effects of cereals postprandial measures with extensive sampling are needed. Individuals with different health and metabolic status seem to respond differently to whole grain interventions and responsive metabolic phenotypes need to be defined to improve and tailor dietary guidelines in prevention of cardiometabolic disease.

2 AIMS & OBJECTIVES

The overall aim of this thesis was to evaluate whether replacing refined grains with whole grains in habitual diets improves metabolic markers, appetite control, and body weight in individuals with elevated cardiometabolic disease risk (individuals with overweight, obesity or type 2 diabetes). The aim was further to explore the potential role of metabolic markers, gut microbiota and appetite in diet-induced weight loss with focus on wholegrain rye. Findings from these investigations may contribute to the development of evidence-based dietary guidelines tailored to populations at increased risk for cardiometabolic diseases and the development of healthy cereal foods.

Specific objectives:

1) To evaluate the effects of largely intact compared with finely milled whole grains on blood glucose control in adults with type 2 diabetes.

2) To evaluate whether subjective appetite measures in free-living conditions align with those measured under controlled clinical conditions.

3) To investigate effects of substituting refined wheat with wholegrain rye foods, on day-long postprandial responses of incretins, ghrelin, glucose, inflammatory markers and subjective appetite.

4) To evaluate the effects of 12-week hypocaloric wholegrain rye-based versus a refined wheat-based diets on body weight and body fat.

5) To examine diet-induced changes in gut microbiota, short-chain fatty acids, metabolic risk markers, and appetite, and explore them as potential determinants of weight loss.

3 BACKGROUND

3.1. Cereals, whole grains, and their role in health

Cereals are carbohydrate-rich foods, as approximately 75% of dry weight is composed of carbohydrates, mainly derived from the starchy endosperm [43]. However, cereals are also an important source of plant-based proteins, Vitamin B, minerals and the most important food source of dietary fiber, globally [14,15]. Whole grain intake has been inversely associated with body weight and body fat mass in epidemiological studies[16,44]. Furthermore, observational data consistently show a reduced risk of coronary heart disease, colorectal cancer, type 2 diabetes, and all-cause mortality when comparing higher with lower intakes of whole grain [13]. WHO dietary guidelines, along with many national guidelines, advocate for increasing whole grain intake and replacing refined grains with whole grains as part of a healthy and sustainable diet. Sweden's food-based dietary guidelines have long emphasized the importance of grains in the diet and that grain-based foods should be consumed as whole grains [45].

There is no international consensus on the definition of whole grains or whole grain products, with several multi-stakeholder initiatives and government agencies using different definitions. This lack of a global definition complicates research and interpretations of health benefits of whole grains. The international initiative - *Whole Grain Initiative* with experts from academia, industry *and* government agencies have defined whole grains as “Whole grains shall consist of the intact, ground, cracked, flaked or otherwise processed kernel after the removal of inedible parts such as the hull and husk; all anatomical components, including the endosperm, germ, and bran must be present in the same relative proportions as in the intact kernel [46]. Moreover, the global *Whole Grain Initiative* emphasizes that foods labeled whole grain must contain at least 50% of whole grain by dry weight, but foods with 25-50% may still make a front-pack claim that the product contains whole grain.

Food and Drug Administration (FDA) in the US was the first national agency to require whole grain foods to contain ≥51% whole grain ingredients by weight for front-pack-labeling in 2001 [47]. The UK followed in 2003, however national definitions vary widely across Europe, with Germany stating at least 90% being whole grain, while Denmark and Sweden required 50% of the food dry matter constituted of whole grains.

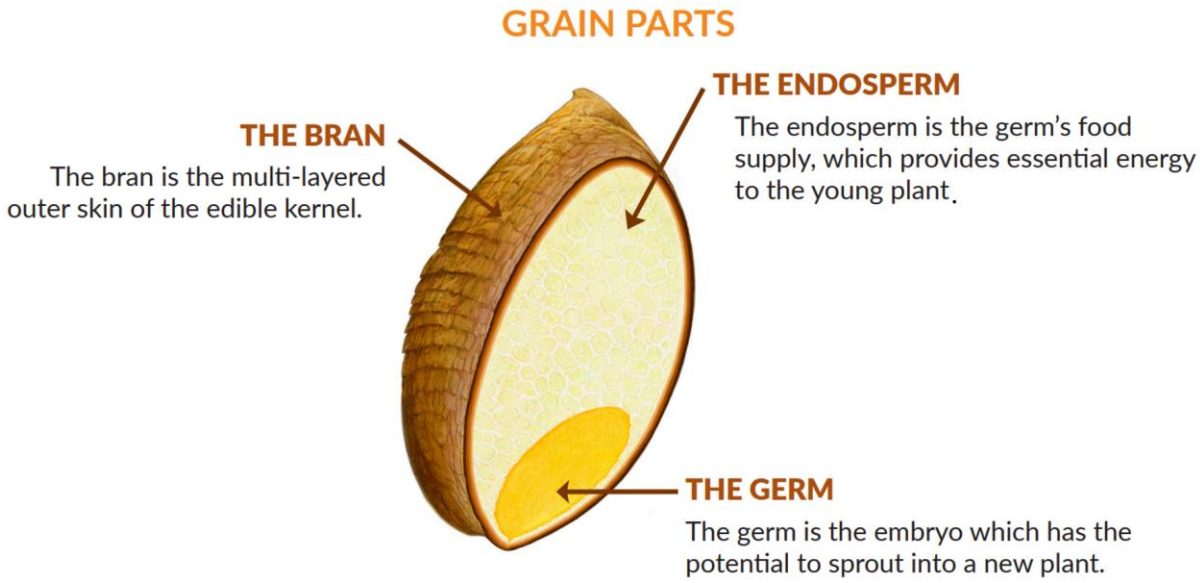

Figure 3.1.A. Anatomy of generic grain, edible parts illustrated. Modified illustration from *The Whole Grains Council* [48].

Overall, national dietary guidelines for whole grain and dietary fibre intake are not met. In the Nordic countries, only 16% to 35% of the population, depending on gender and country, meet the recommended whole grain intake [49]. However, Denmark has seen significant success in boosting whole grain consumption through *the Danish Whole Grain Partnership*, a public-private initiative, with over 50% of Danes now meeting the recommendations [50]. In the USA, 98% of the population falls short of the recommendation that at least 50% of grains consumed should be whole grains [51]. This is also reflected in the dietary fibre intake, where USA and Canada had the lowest average daily intakes (14-18g/day) when compared with European countries, New Zealand, and the UK [52]. While intakes were higher on average for European countries, no single country met the national recommendations for dietary fibre intake on average [52].

Whole grain consumption varies widely across the globe, both in terms of the types of grains and grain products consumed. Globally, rice is the most consumed cereal, followed by wheat and maize [15]. In southern and central Europe, wheat dominates cereal consumption, while rye-based cereals are more commonly consumed in northern Europe. In countries like Germany, the Baltic states, and Scandinavia, rye foods are traditionally made with a high proportion of whole grain [49,53].

The nutrient composition of whole grains significantly differs from refined grains, where the bran and germ are removed. **Figure 3.1.B** illustrates the relative nutrient composition of wholegrain wheat flour compared to white refined wheat flour. Nutrient content and physiological properties also vary considerably among wholegrain cereals. Wheat and barley are rich in phenolic compounds, oats and barley rich in β-glucans, and rye has the highest amount of arabinoxylans [33]. Wholegrain rye is the cereal with the highest dietary fiber content and has consistently demonstrated satiating effects compared to refined alternatives [20–24].

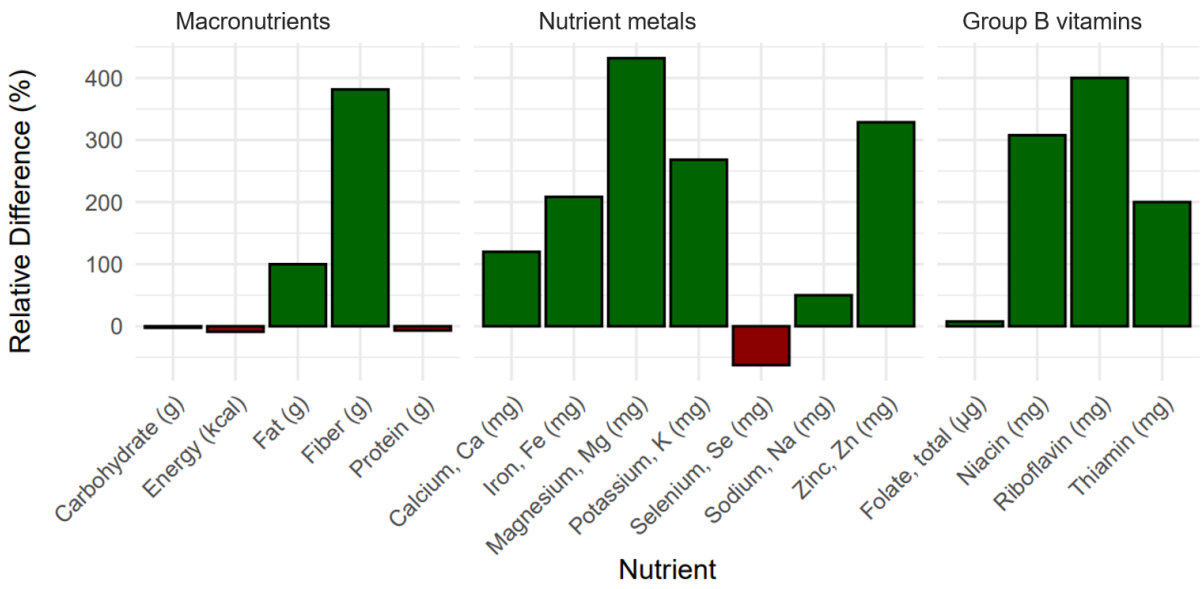

Figure 3.1.B. Wheat flour, whole grain and refined grain flour relative nutrient composition (%). Data from USDA [54].

3.2. Metabolic effects of whole grains -emphasis on rye

3.2.1. Glycemic control

Commonly consumed refined cereals derived from corn, rice, and wheat, elicit a high glycemic response and substitution with whole grain alternatives may improve glycemic control and mitigate the risk of various non-communicable diseases [20,55,56].

Wholegrain cereals have consistently been reported to improved postprandial glycemia compared to their refined counterparts [20,31,55,56]. Oats and barley contain high levels of the viscous fiber β-glucan, that has repeatedly been reported to improve measures of glycemia [57–59]. The European Food Safety Authority (EFSA) has authorized a health claim for postprandial glycemia, stating that foods containing at least 4 g of β-glucans from oats or barley per 30 g of available carbohydrates qualify for the claim [60].

While the beneficial effects of oats and barley on postprandial glycemia are known to be attributed to β-glucans, our understanding of wholegrain rye and the mechanisms responsible for its possible blood glucose-lowering effects remains less explored. A possible factor could be arabinoxylan induced viscosity, slowing gastric emptying and subsequent available glucose in the small intestine. whole grain rye like other whole grain cereals comprises compartmentalization of starch, affecting starch and protein interactions, that may impact digestion rate [35,61]. Epidemiological studies suggest that a higher dietary intake of wholegrain rye relative to wholegrain wheat can reduce the risk of type 2 diabetes [32].

Some intervention studies have demonstrated improved acute meal responses when comparing commonly consumed refined wheat cereals with wholegrain rye alternatives [31]. Properties of rye cereals on glycemic control have not been studied to the same extent as wheat and oats,

calling for more trails evaluating effects of rye foods [56] . In three studies by *Rosén et al*. [24,62,63] and one study by *Goletzke et al*. [64] postprandial glycemia was reduced after consumption of bread made of wholegrain rye flour compared with refined what flour. Participants in all these studies were young, healthy individuals, BMI <25 kg/m^2. *Hartvigsen et al*. showed reduced glucose AUC after consumption of wholegrain rye porridge versus semolina porridge in overweight individuals with metabolic syndrome [21]. Similarly, *Lee et al*. found comparable results in lean individuals consuming rye porridge [23]. However, these studies focused on the acute effects of meals composed entirely of rye foods. In contrast, a few studies have investigated the effects of replacing habitual cereals with wholegrain rye and reported no significant differences in postprandial glycemia [31]. While whole grains generally show reduced postprandial glycemia compared to their refined counterparts, rye foods made from rye endosperm, have shown potential to improve glycemic and insulinemic profiles when compared to wheat endosperm foods [62].

Dietary interventions targeting postprandial glucose and long-term fasting glucose are especially crucial for populations exhibiting signs of impaired fasting glucose and glucose intolerance. The pathophysiology behind impaired insulin sensitivity and glucose homeostasis is complex and varies among individuals [65], with clinical markers such as fasting glucose, fasting insulin, and homeostatic model assessment for insulin resistance (HOMA-IR) commonly used to monitor these processes [66].

The need for individuals with diabetes to conveniently monitor blood glucose levels throughout the day has driven significant technological advancements. Traditionally, glucose monitoring has relied on finger-pricking, typically 4 to 10 times per day [67]. However, the development of continuous glucose monitors (CGMs), which automatically measure blood glucose every five to fifteen minutes (depending on the manufacturer), has drastically improved glucose management for diabetic patients [68]. Beyond clinical use, CGMs have also enabled resource-efficient data collection in nutrition research, providing large datasets that enhance our understanding of the metabolic response to diet and food. In brief, a CGM system consists of three main components: a sensor, transmitter, and receiver. The sensor, the most advanced part of the system, is a thin metal wire (even thinner than a needle) inserted just under the skin into the subcutaneous fat layer, typically on the abdomen or arm (**Figure 3.2.1**). Recent evaluations of factory-calibrated CGM sensors have shown good agreement with traditional capillary point-of-care measures [69–71].

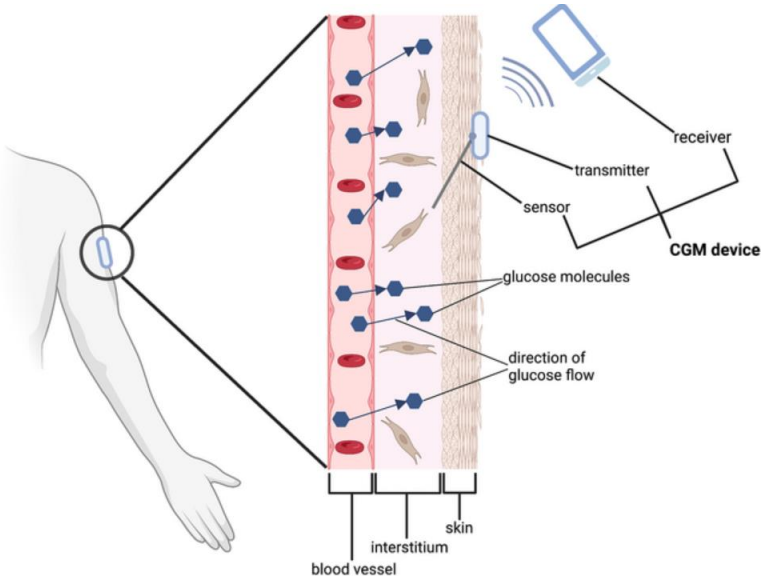

Figure 3.2.1. An illustration showing the flow of glucose from the bloodstream into the interstitial fluid, where it is detected by the continuous glucose monitoring (CGM). Reproduced from *Brar et al.* [72] with permission from John Wiley and Sons.

In nutrition research, common measures of postprandial glucose include mean glucose concentrations and the incremental area under the glucose curve (iAUC). Peak glucose concentration is also frequently reported. However, the extensive data from CGMs has enabled more detailed assessments of postprandial glycemic variability. Some of the most widely used metrics for this include the standard deviation of mean glucose (SD), coefficient of variation (CV), mean amplitude of glycemic excursions (MAGE), and the continuous overall net glycemic action index (CONGA).

3.2.2. Subjective appetite

Appetite is commonly defined in nutrition research as the psychological desire for foods or beverages. Factors influencing appetite include sensory responses to the sight, taste, smell, and even sound associated with food. Additionally, behavioral and social factors can influence these sensory responses and alter the perception of food [73,74]. In parallel, satiation is defined as the process that leads to the termination of eating and satiety inhibits further eating [73]. The appetite system directly influences energy intake and is closely tied to body composition and obesity [8]. Studying foods and dietary strategies that reduce hunger and enhance feelings of fullness is crucial to understanding the factors driving the development of overweight and obesity.

After food ingestion, a series of physiological and endocrine processes are triggered to signal satiation and eventually terminate eating, as illustrated by *Blundell et al.* in **Figure 3.2.2**. Sensory impressions and subjective sensations of meals and foods are challenging to quantify, and gastrointestinal hormones involved in central appetite regulation have been suggested as “gold standard”. In lean individuals, concentrations of these gut hormones have indeed shown

correlations with hunger and fullness ratings [75]. While these gut hormones serve as objective biomarkers, they are expensive to analyze and difficult to measure as they degrade quickly, and the role of these hormones in short-term appetite, subsequent energy intake and body weight control is not well established [8,29]. Subjective appetite sensations, traditionally measured by ratings of hunger and fullness using 100 mm visual analogue scales (VAS), provide valuable information and are highly reproducible within individuals [76,77]. Many studies have used VAS to measure postprandial appetite with test meals provided in a research clinic and shown good validity [73,78]. That said, the prevailing consensus among most researchers in the field is that no "gold standard" for appetite measurements exists to date.

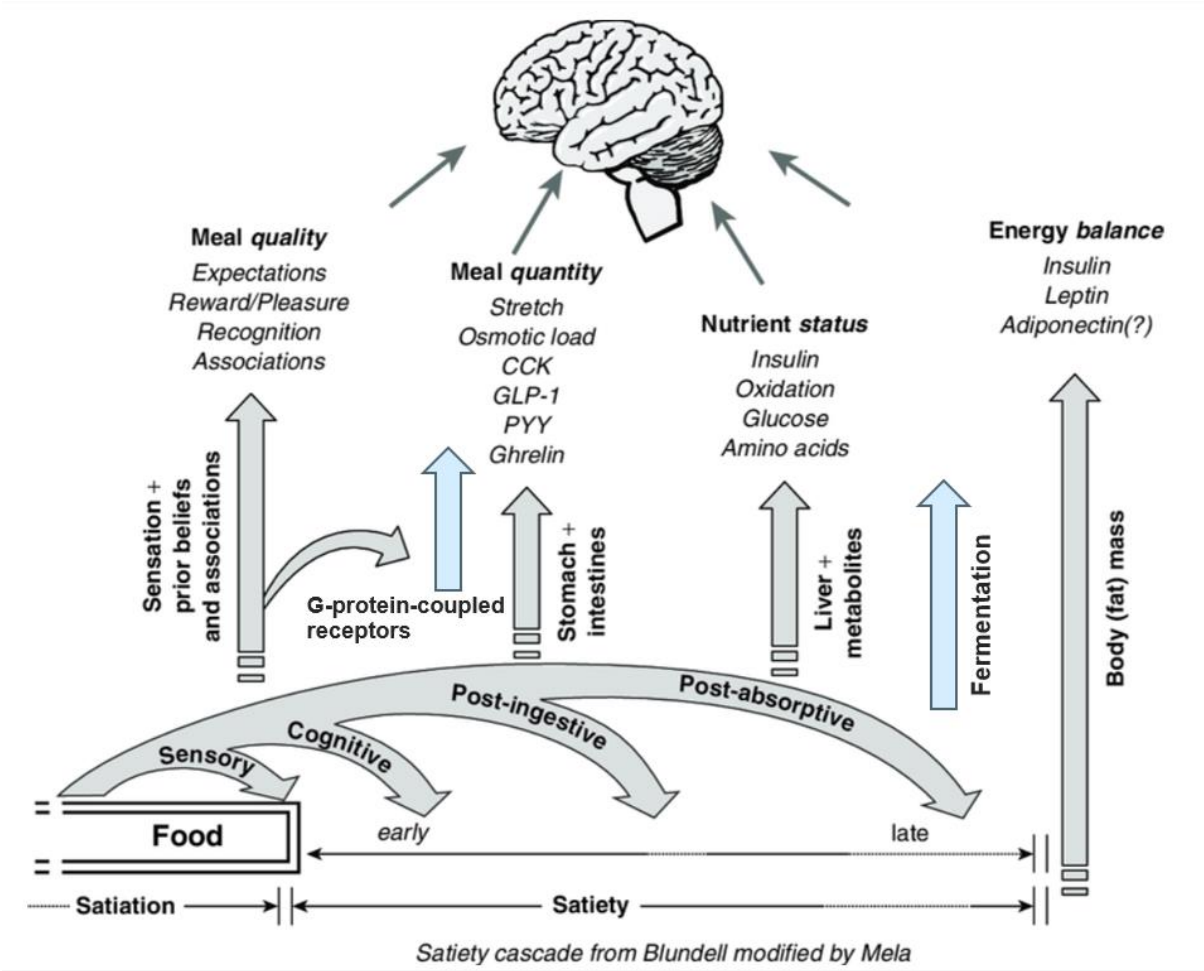

Figure 3.2.2. The satiety cascade. Modified reproduction from *Blundell et al. 2010* [73], with permission from John Wiley and Sons. Abbreviations: CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

Dietary interventions have consistently show increased satiety after consuming wholegrain rye compared to refined wheat [21–24]. In all studies, acute meal responses were tested, and all test-meals consisted exclusively of wheat or rye foods. In two similar studies *Isaksson et al.* showed increased satiety in the 4-h postprandial phase when rye foods contributed two thirds of the total energy content [79,80]. In the first study *Isaksson et al.*, showed sustained appetite-suppressing effects of a whole rye-kernel vs milled rye-kernel breakfast also after a standardized lunch (not including rye) [79]. Some individuals seem more susceptible to overeating, and inter-individual

variations in appetite control may be of importance [25,26]. *Drapeau et al.* studied behavioral phenotypes in obesity and identified a "low-satiety phenotype" characterized by an impaired ability to detect appetite sensations [81]. Approximately 10 % of individuals under weight loss treatment were classified as the low-satiety phenotype, showing a blunted cortisol response to test meals, indicating dysregulated hypothalamic activity. When evaluating appetite regulating properties of wholegrain foods, altered appetite signaling in overweight and obese individuals should be considered. Notably, only one of the above-mentioned trials focused on individuals with overweight and obesity, showing reduced hunger but no differences in fullness or prospective consumption following a breakfast of 100% rye porridge [21].

Recently, appetite responses to consecutive wholegrain rye- versus refined wheat-based meals were investigated in individuals with overweight and obesity [37]. In this 12-week hypocaloric intervention, appetite was assessed in a free-living setting at baseline, mid-intervention, and 12-week follow-up. Overall, no consistent differences in appetite responses between the rye and wheat-based meals were observed, and the reported differences in weight loss could not be attributed to appetite. Studies in individuals with overweight and obesity are limited, as are studies examining subjective appetite ratings of mixed meals or complex diets containing wholegrain rye foods.

3.2.3. Appetite-regulating hormones

Objective biomarkers of appetite and satiety are important complements to subjective appetite ratings [8]. Ghrelin, an orexigenic hormone released from the stomach, rises during fasting, while CCK (cholecystokinin), GLP-1 (glucagon-like peptide-1), PYY (peptide YY), and GIP (glucose-dependent insulinotropic peptide) are anorexigenic hormones that increase in response to food intake. These peptides play a key role in appetite regulation through the gut-brain axis and provide valuable insights into the satiating properties of meals and foods [8,29,82]. Incretins, particularly GIP and GLP-1, play a crucial role in stimulating insulin and glucagon secretion by the pancreas. Consequently, incretin-based glucose-lowering medications, especially GLP-1 receptor agonists, have proven effective and are widely used in the treatment of type 2 diabetes [83,84]. GLP-1, identified as a central peptide in regulating hunger, and satiety, has led to the widespread use of GLP-1 receptor agonists in obesity treatment, with recent dual GLP-1/GIP agonists achieving weight loss comparable to bariatric surgery [9].

As key components of appetite regulation, investigating the postprandial response of gut peptides to intervention foods and diets is essential. Wholegrain cereals in general [12], and rye cereals in particular [21–24], have consistently been shown to enhance satiety and reduce hunger compared to their refined counterparts. However, studies examining the effects on appetite-regulating hormones remain scarce, and the findings are inconclusive [21,23,24,30].

In 1999, *Juntunen et al*. [30] showed lower postprandial GLP-1 and GIP concentrations in healthy subjects consuming wholegrain rye kernel bread compared with refined wheat bread. Interestingly the same group studied structural differences and demonstrated that traditional rye bread resulted in a lower GIP response compared to endosperm rye bread, with a trend towards

lower glucose concentrations [85]. The traditional rye bread, characterized by closely packed starch granules, differed significantly from refined endosperm rye bread in total fiber content, yet produced similar insulin and C-peptide responses. This suggests that less insulin is required to regulate postprandial GIP and glucose levels. More recently, *Hartvigsen et al.* [21], showed lower GLP-1 response to porridge made of rolled wholegrain rye flakes versus semolina porridge in individuals with overweight and obesity, while postprandial ghrelin levels did not differ between the two meals. *Heinonen et al.* [86], observed different response among lean participants and those with obesity, where lean individuals reduced ghrelin concentrations significantly after consuming wholegrain rye vs refined wheat bread, a contrast not observed in participants with obesity. Similarly, *Rósen et al*. reported reduced postprandial ghrelin following wholegrain rye consumption compared to refined wheat foods in normal-weight individuals [24,62]. Studies examining appetite-regulating hormones in response to whole grains, particularly rye foods, are limited, and variations in study design and populations make comparisons challenging.

3.2.4 Inflammatory markers

Observational data have shown that higher intake of whole grains was associated with lower CRP concentrations independent of demographic, lifestyle, and dietary variables [87,88]. *Taskinen et al.* reported in an elderly population that every 50 g/d higher whole grain intake was associated with 0.12 mg/L lower CRP, while 50 g/d increase in refined grain was associated with 0.23 mg/L higher concentration [87]. Also, dietary interventions have shown beneficial effects of wholegrain foods on inflammatory markers [89]. Effects of whole grains on subclinical inflammation seem to be more pronounced in individuals with overweight or obesity [90].

In a systematic review of controlled trials, *Milesi et al. [89]* reported that the consumption of whole grain foods resulted in a reduction of at least one inflammatory marker. However, only 3 out of 31 studies included instructions for participants to maintain their body weight during the intervention, and just one study adjusted for weight in the analysis [91].

Effects of whole grains on subclinical inflammation may not be homogeneous across different type of grains varying in amounts and type of fibers and polyphenol [89,92]. Three studies of 12-week interventions with wholegrain rye foods have demonstrated reductions in inflammatory markers. Two studies showed reductions in CRP levels when compared with 12 weeks of refined wheat foods [37,93]. Additionally, one study showed a decrease in circulating IL-6 and adipose tissue-specific inflammation markers when compared to whole meal oat and wheat [94]. Interestingly, participants in these interventions were all overweight or obese, confirming findings from *Milesi et al.* [89]. These rye-based interventions seem to reduce markers of inflammation without changes in body weight [93,94], or when body weight changes were adjusted for [37].

Novel inflammatory markers glycoprotein N-acetylation (GlycA and GlycB) and supramolecular phospholipid composite peak (SPC) detected by nuclear magnetic resonance (NMR) spectroscopy [95], have recently been suggested to better reflect low-grade system inflammation than traditional inflammatory markers such as CRP and IL-6 [96]. GlycA has gained most attention as the most promising candidate, with elevated levels reported in individuals with metabolic syndrome and type 2 diabetes [97]. Additionally, GlycA has been associated with an increased risk of type 2 diabetes, cardiovascular disease, and all-cause mortality [96]. However,

intervention studies evaluating dietary effects, particularly the impact of whole grains on inflammatory markers, rarely report GlycA.

Overall, established inflammatory markers like CRP and TNF-alpha have rarely shown significant changes in the postprandial phase, while circulating IL-6 has exhibited some differences postprandially [98], studies assessing diet-induced inflammatory responses are scarce. Recently, GlycA has been identified as a promising biomarker for assessing diet-induced inflammatory responses in the postprandial phase [99].

3.3. Wholegrain rye and body weight management

Wholegrain rye has physiological properties and demonstrated appetite regulating effects that makes rye-based foods interesting for weight management. Two cross-over trials evaluated weight change over 4-weeks of wholegrain rye and refined wheat but found no difference [100,101]. Another two cross-over trials compared wholegrain rye and wholegrain wheat but found no difference in body weight [102,103]. None of these studies were designed for weight loss and the duration of these cross-over trials may not be sufficient to assess effects of rye foods on body weight. However, two studies have investigated effects on body weight when habitual cereals were replaced with either wholegrain rye or refined wheat foods [37,104]. In a 6-week, 3-arm parallel intervention, *Suhr et al*. demonstrated a 1.1 kg body weight loss in the whole grain rye-group, compared to a 0.6 kg weight loss in the wholegrain wheat-group and a 0.2 kg weight gain in the refined wheat-group [104]. It is important to note that the primary focus of the study was to investigate gastrointestinal symptoms and gut microbiota composition following wholegrain rye and wheat interventions. In contrast, *Iversen et al*. conducted a 12-week hypocaloric intervention comparing wholegrain rye and refined wheat, specifically aiming to evaluate differences in body weight and composition [37]. *A* greater body weight loss of was reported for the rye-group (2.9 kg) compared to the wheat-group (1.8 kg), with similar differences observed in body fat reduction. This was the first intervention study designed to investigate differences in weight loss where greater weight loss with wholegrain rye foods compared to refined wheat foods as a replacement for habitual cereals was demonstrated.

Several factors may contribute to the observed weight loss following high intake of wholegrain rye foods, including satiating properties. Gut microbiota composition has also been suggested as a determinant of weight loss in high fibre dietary interventions [38,39]. Specifically, individuals with high baseline abundance of Prevotella have shown greater weight loss following a wholegrain rye-based diet [105]. However, most studies show no associations with baseline microbiota and weight loss [40,41]. *Iversen et al*. found no associations with baseline microbiota; however, the rye-based diet induced changes in short-chain fatty acids that may influence appetite regulation [42]. As the cereal with the highest fiber content, rye may induce weight loss through increased ileal energy excretion. Recently, it was shown that consuming wholegrain rye bread, compared to refined wheat bread, decreased energy absorption in the small intestine, consequently nutrients available for fermentation in the large intestine increased [106]. To date, few studies have demonstrated that wholegrain rye interventions induce greater weight loss compared to other whole grains or refined grain alternatives.

3.4. Wholegrain rye, gut microbiota and SCFA

Most of the human microbiota resides within the intestines and is shaped by factors such as birth method, infant feeding, lifestyle, medications, and host genetics [107]. Among these, lifestyle is modifiable, and growing interest in nutrition research focuses on the links between diet, the gut microbiome, and body weight changes. Already 15 years ago, *Turnbaugh et al.* demonstrated distinct differences in lean and obese monozygotic twin pairs with reduced bacterial diversity, phylum-level changes and alterations in related metabolic pathways [108].

It is widely recognized that the gut microbiota has complex associations with the onset and development of obesity [108]. Changing the microbiota composition through diet, lifestyle, probiotics, or fecal transplantation shows potential for obesity management. However, results in humans are still inconsistent [109]. Additionally, prebiotic effects of high fiber diets have been suggested to alter gut microbiota and mediate weight loss. As metabolites of microbial fermentation and key signaling molecules in appetite regulation [110–112], SCFAs are valuable to monitor alongside microbial changes in response to whole grain-rich dietary interventions and their associations with body weight changes (**Figure 3.4**).

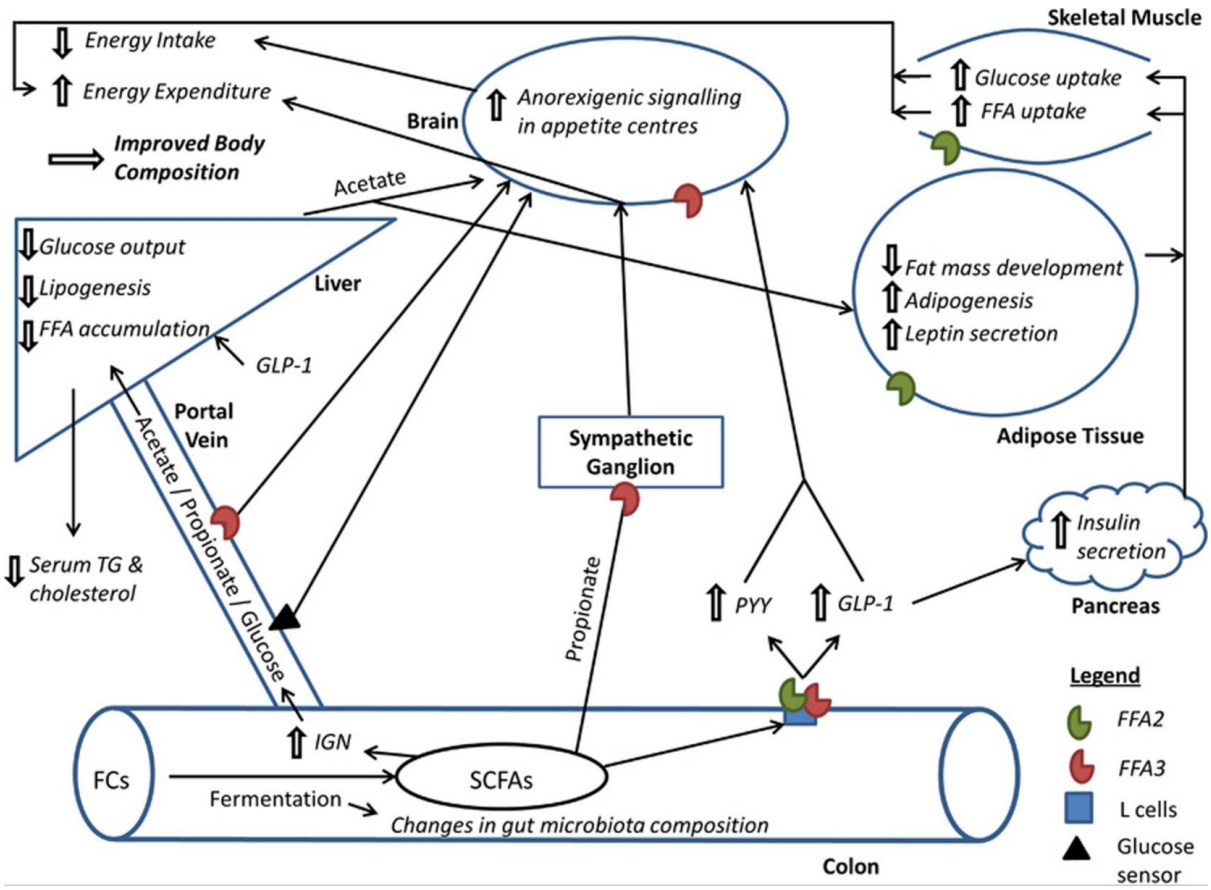

Figure 3.4. An overarching model for the beneficial effects of colonic SCFA production on appetite regulation and energy homeostasis. Reproduced from *Byrne et al. 2015* [111] , with permission from Springer Nature. FCs, fermentable carbohydrates; FFA, free fatty acids; FFA2, free fatty acid receptor 2; FFA3, free fatty acid receptor 3; GLP-1, glucagon like peptide-1; IGN, intestinal gluconeogenesis; PYY, peptide YY; SCFAs, short chain fatty acids; TG, triglyceride.

Few dietary interventions comprising wholegrain foods have been able to demonstrate alterations of the gut microbiota [41,113–116]. The limited impact on gut microbiota may be due to several reasons. Several of these studies were designed for other primary objectives and thus have insufficient power to evaluate effects on the microbiota. Some studies substituted participants' habitual cereals with intervention foods, which may not have induced significant shifts in individuals with already high whole grain and fiber intake as part of their habitual diet. Additionally, the broad and inconsistent definition of whole grains in different countries and in research may obscure their effects on gut microbes. These factors contribute to the variability of results in whole grain interventions.

A few dietary interventions with wholegrain foods [42,116,117] and supplementation with resistant starch [115,118] have shown effects on microbiota composition. However, there is very limited data on wholegrain rye specifically. *Gråsten et al.* [119] reported no alteration of the gut microbiota in healthy postmenopausal women consuming high fibre rye bread vs white wheat bread as part of a complex diet. Neither did *Vuholm et al.* [120] when effects on gut microbiota following 6-weeks of a wholegrain rye-based diet was evaluated in participants with overweight. However, some dietary interventions providing wholegrain rye foods have reported alterations of the gut microbiota.

In a crossover trial, patients with irritable bowel syndrome (IBS) showed increased *Flavonifractor* on genus level consuming wholegrain rye bread for four weeks [117]. More pronounced changes in the fecal microbiota were observed with a modified rye bread lower in fructans and low molecular weight fibers [117]. *Eriksen et al.* [103] analyzed microbiota composition after 8 weeks with wholegrain rye and wheat foods in men with overweight and signs of metabolic syndrome. Abundance of Bifidobacterium increased after the wholegrain rye intervention and Clostridium were reduced at genus level after wholegrain rye compared to wholegrain wheat. In a recent 12-week trial, cereal intervention foods contributed ~30% of daily caloric intake, with the rye-group showing increased Agathobacter and UCG_002, while Anaerotruncus, Anaerofilum, and Holdemania decreased [42]. Plasma acetate and butyrate concentrations were higher at the 6-week follow-up and butyrate higher at the 12-week follow-up in the rye-group compared with the wheat-group. *Vetrani et al.* [121] observed increased propionate after 12 weeks of mixed wholegrain sources in participants with metabolic syndrome and *Damen et al.* [122] reported increased fecal butyrate and trends toward higher acetate in normal-weight individuals consuming arabinoxylan-fortified bread.

The use of different analytical methods for SCFA and microbiota analyses complicates comparisons across studies. Additionally, studies reporting diet-induced changes in SCFAs report measurements derived from both fecal samples and plasma. *Müller et al. [123]* found plasma, but not fecal SCFAs inversely correlated with BMI and positively correlated with GLP-1 and insulin sensitivity, indicating an important distinction between plasma and fecal SCFAs and their associations with metabolic markers.

3.5. Inter-individual variability and determinants of diet-induced weight loss

Substantial inter-individual weight loss variability has been observed in hypocaloric dietary interventions [36,124,125]. There are many causes and possible mediators of differences in diet-induced weight loss between individuals. Beyond recognized factors like age, gender, physical activity, medication, and diet adherence, additional inter-individual differences—such as genetic factors, gastrointestinal peptides, metabolic capacity, thermogenic response to food, and the gut microbiome—are also suggested to influence adaptation to energy alterations and individual weight loss capacity [36]. *Gerrits et al.* [125] showed that the rate of weight loss during a 900 kcal/day meal replacement program varied more than two-fold between individuals, even after controlling for factors such as diet adherence, initial body weight, physical activity, gender, and medical conditions. *Dent et al.* [126] showed a 3-fold weight loss variability after 6 weeks of meal replacement in a cohort of 1800 women, after adjustment for recognized factors mentioned above (**Figure 3.5.A**).

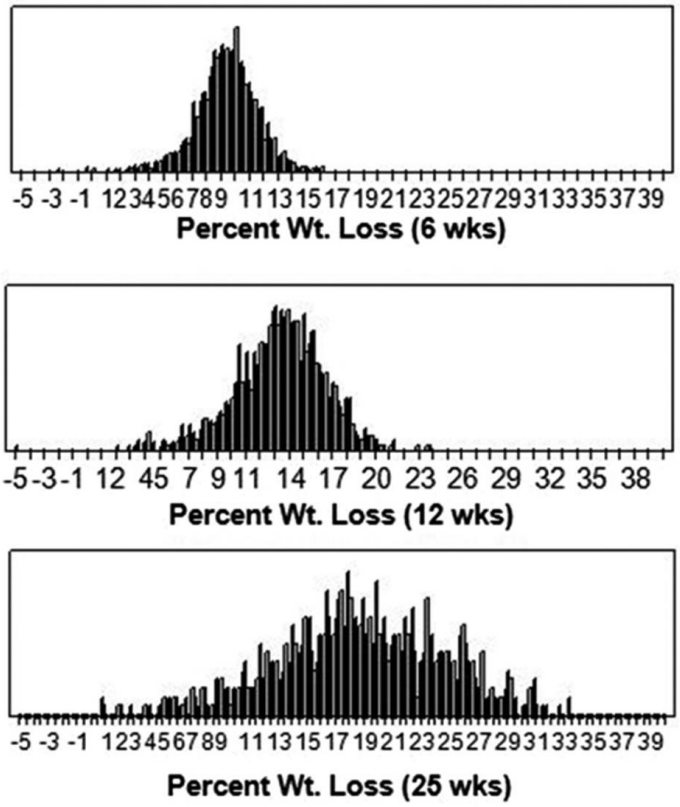

Figure 3.5.A. Inter-individual variability in response to 6, 12, and 26 weeks of meal replacement in 1800 women 30-60 years of age. Reproduced from *Dent et al. 1999* [126], with permission from John Wiley and Sons.

Recently, metabolic factors and gut microbiota have been discussed in relation to successful weight loss. In two large multicenter dietary trials, fasting blood glucose and insulin were shown to predict weight loss following high vs low glycemic load/whole grain rich diets [127]. In the SHOPUS study [128] 181 adults with increased waist circumference were randomized to either a

New Nordic Diet or an average Danish diet. Prediabetic individuals, defined by elevated fasting glucose lost 6 kg more on the New Nordic Diet compared to those on the Danish diet, while normoglycemic participants lost an average of 2.2 kg more. In the DiOGenes trial [129] adults with overweight or obesity who had lost 8% of their body weight in the initial phase were included. A total of 772 participants with an average BMI of 34, from eight European countries, were randomized to 26-week diet interventions varying in protein content and glycemic load. When stratified by glycemic status, prediabetic individuals regained an average of 5.8 kg more on the high–glycemic load diet compared to the low–glycemic load diet, while normoglycemic participants regained an average of 1.4 kg.

Baseline gut microbiota and enterotypes have been suggested to predict and mediate high fiber diet induced weight loss [38,39]. Enterotypes are defined as stable microbial community compositions that are not determined by age, gender, body weight, or nationality [130]. The three distinct enterotypes are: type 1, characterized by high levels of Bacteroides; type 2, with a high abundance of Prevotella; and type 3, dominated by Ruminococcus (**Figure 3.5.B**). Recently, a type 4 has also been suggested, rich in specific Bacteroides [131]. Evidence suggests that long-term diet, rather than demographic factors, plays a key role in shaping enterotypes [132]. *Christensen et al.* showed that individuals classified as Prevotella dominant at baseline lost more body weight after 6 weeks of a wholegrain rye rich dietary intervention [105]. These are relatively small studies with 15 and 28 participants in the intervention groups define by high Prevotella respectively [38,105]. Most dietary interventions rich in wholegrain foods show no associations with baseline microbiota genus or enterotypes and weight or body composition changes [37,40,41].

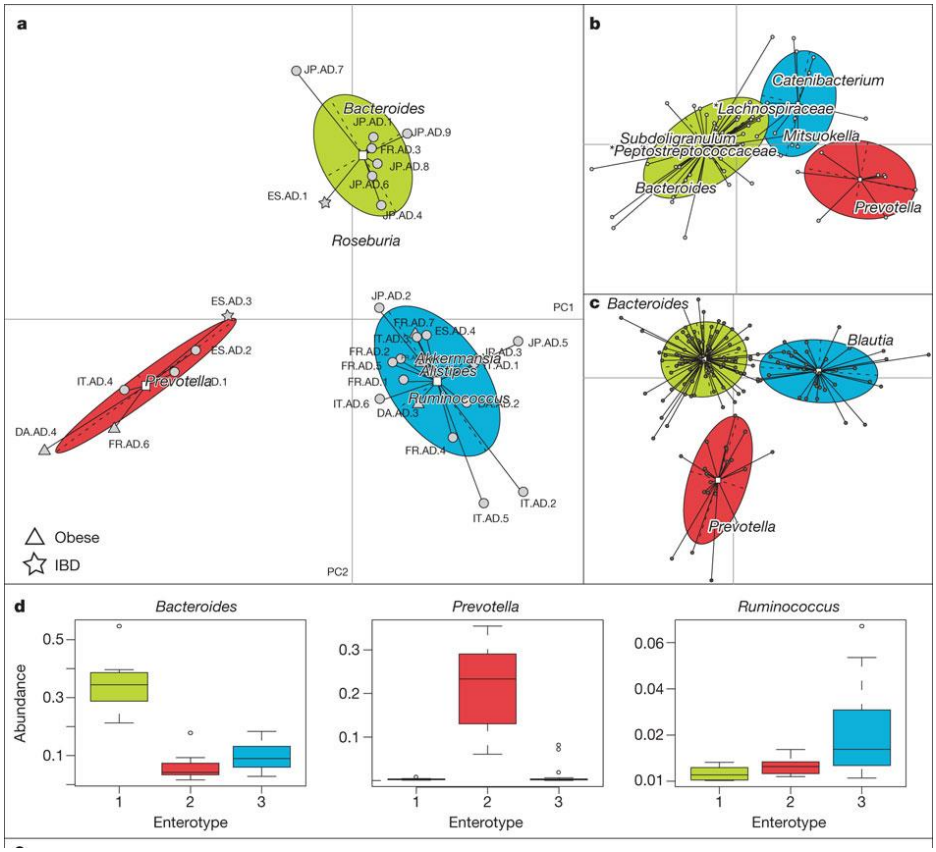

Figure 3.5.B. Enterotypes of the human gut microbiome. Principal component analysis and clustering, of the genus compositions of 33 metagenomes estimated by mapping the metagenome reads to 1511 reference genome sequences. Modified and reproduced from *Arumugam et al. 2011* [130], with permission from John Wiley and Sons.

SCFAs have been proposed as mediators of improved insulin sensitivity by reducing fatty acid synthesis and increasing lipolysis in skeletal muscle [133]. Additionally, SCFAs may influence pancreatic function by regulating insulin secretion, as propionate supplementation has shown effects on glucose-stimulated insulin secretion [134]. Acetate has also been found to reduce plasma insulin levels in rat pancreas tissue [135]. However, studies on the direct effects of SCFAs on skeletal muscle metabolism and pancreatic function in humans remain limited. Dietary intervention studies inducing changes in insulin and fasting glucose could explore associations with baseline SCFA levels.

4 METHODS

4.1. Hypotheses and research strategies

The overall hypothesis investigated was that replacing refined grains with wholegrain rye in habitual diets would improve metabolic markers, appetite control, and body weight in individuals at elevated metabolic risk and that more intact whole grain structure provides additional benefits for blood glucose control compared to finely milled grains.

Furthermore, effects of whole grain intake on metabolic risk markers and body weight are hypothesized to be more pronounced in individuals with elevated cardiometabolic disease risk, with metabolic status and gut microbiota mediating these effects. The specific hypotheses investigated were:

1) *Wholegrain processing, specifically milling, impairs glycemic control in adults with type 2 diabetes*. To test this hypothesis, a 2-week cross-over trial comparing diets with finely milled versus largely intact wholegrains was conducted (**Paper I**).

2) *Subjective appetite measured under controlled clinical conditions differ from appetite responses under free-living conditions and gut hormones reflect distinct aspects of appetite regulation that self-reported measures may not fully capture*. We conducted a 5-way cross-over trail to address this hypothesis (**Paper II**) and to assess whether gut hormones, as objective biomarkers of appetite, differed between wholegrain rye and refined wheat-based diets (**Paper III**). We also explored day-long glycemic response and diet-induced inflammation following these diets (**Paper III).**

3) *Wholegrain rye foods will contribute to greater body weight reductions than corresponding refined wheat foods through appetite regulating properties. The effects may vary substantially with individual metabolic status and host gut microbiota*. We investigated this in the RyeWeight2 study (**Paper IV**), with the same design as the previous RyeWeight1 study, where our research-group found that wholegrain rye, as part of a 12-week hypocaloric diet was more effective in reducing body weight and fat mass, with considerable inter-individual variation, despite no observed effects on subjective appetite (27).

Table 4.1. Overview of the studies included in the thesis.

	the Whole-grain milling & glycemia trial (Paper I)	the VASA-home study (Paper II & III)	the RyeWeight2 study (Paper IV)
Study design	2 week, cross-over trial, randomized, n = 31	1 day, 5-way cross-over trial, randomized n = 29	12 week, 2-armed, parallel, randomized, n = 255
Inclusion criteria	Males and females 18-80 years, Diabetes type 2	Males and females 30-70 years BMI 27-35 kg/m2	Males and females 30-70 years BMI 27-35 kg/m2
Intervention	Intact whole grains vs finely milled whole grains in the context of habitual diet	Wholegrain rye foods vs refined wheat foods as part of a hypocaloric complex diet	Wholegrain rye foods vs refined wheat foods as part of a hypocaloric complex diet
Primary outcome	Glycemic control	Subjective appetite	Body weight and body fat
Secondary outcomes		Day-long postprandial response of incretins, ghrelin, glucose and inflammation	Metabolic risk markers, SCFAs, gut microbiota composition and determinants of weight loss

4.2. Study designs and study populations

4.2.1. The Whole-grain milling & glycemia trial

The whole-grain milling & glycemia trial, a randomized crossover trial with two dietary interventions of 2-weeks each was conducted with the aim to evaluate the effect of processing, specifically the extent of milling of whole grains on glycemic control and other cardiometabolic risk factors in individuals with type 2 diabetes (**Paper I).** The sample size was calculated to detect a 20% difference in mean postprandial glycemia, measured by iAUC. This estimate, based on a power calculation with an α of 0.05 and a power of 0.80, determined that 28 participants were needed to complete both interventions (**Figure 4.2.1)**. This study was carried out Dunedin, New Zealand.

Participants were between 18 and 80 years of age, diagnosed with type 2 diabetes, and were eligible to participate regardless of medication use or other comorbidities. Pregnancy, lactation or individuals diagnosed with type 1 diabetes were not eligible to participate in the study. After clinical examinations participants were randomized to the order of two interventions of 2 weeks separated by washout. On day 1, baseline fasting blood samples and anthropometric measurements were taken and a CGM sensor applied to the upper arm. On day 14, fasting blood samples and anthropometric measurements were taken and the CGM sensor removed.

All intervention foods were provided, however due to visible differences between intervention products, participants could not be masked. Participants were blinded to CGM data during both interventions and the researcher performing statistical analysis was blinded. Participants were randomly assigned to the intervention sequence using a computer-generated block

randomization protocol. Each participant's assigned sequence was concealed in a numbered, opaque envelope, which was accessed sequentially after obtaining written consent. The primary outcome measure was the incremental area under the glucose curve (iAUC) and secondary outcome included measures of glycemic variability calculated from CGM data.

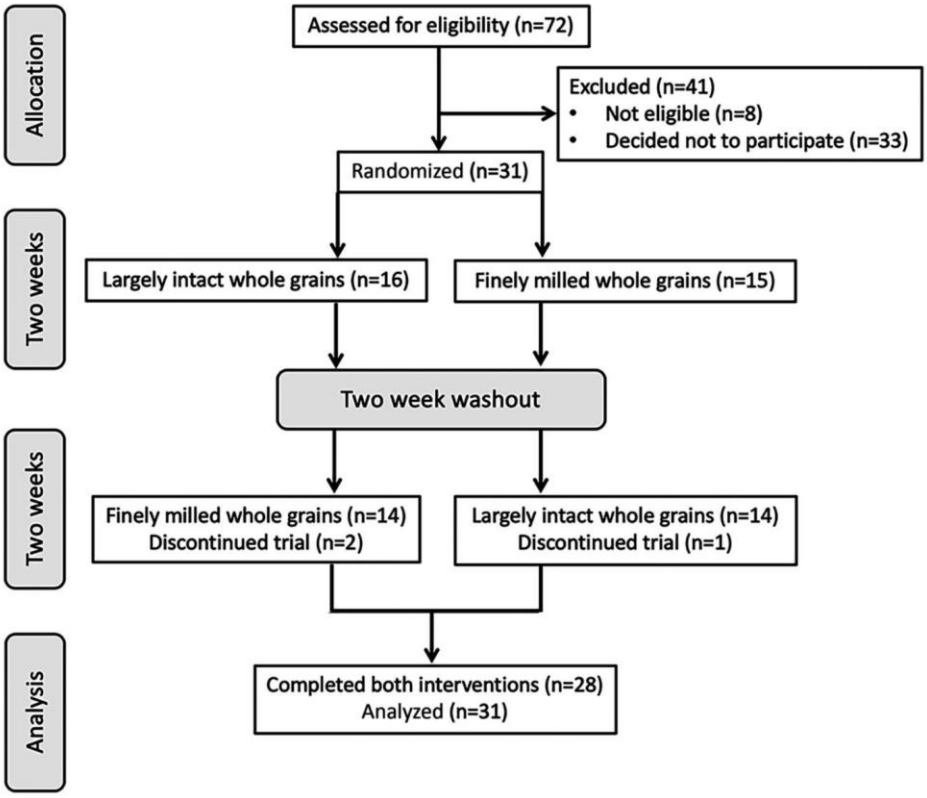

Figure 4.2.1. Flowchart of participants in the Whole-grain milling & glycemia trial (Paper I)

4.2.2. The VASA-home trial

The VASA-home trial was conducted as a 5-way randomized cross-over trial in adults with overweight and obesity. Primarily the study was designed to evaluate the performance of VAS in free-living vs clinic-based settings and to assess subjective appetite response following wholegrain rye and refined wheat-based diets (**Paper II**). Secondary objectives included evaluating the effects of these diets on gut hormones that are relevant in reflecting postprandial appetite response and day-long blood glucose control (**Paper III**). The sample size estimation was calculated to detect 10% within-group differences in appetite ratings in different settings. To allow for secondary comparisons and tolerate a drop-out rate of 30% we aimed to recruit 30 participants (**Figure 4.2.2.A**). Participants continuously answered VAS questions about their perceived appetite (fullness, hunger and desire to eat) from morning 8:00AM to evening 9:00PM while adhering to a strict dietary protocol with either wholegrain rye or refined wheat foods as part of a complex diet. These intervention days were repeated 5 times with one week washout in between (**Figure 4.2.2.B**).

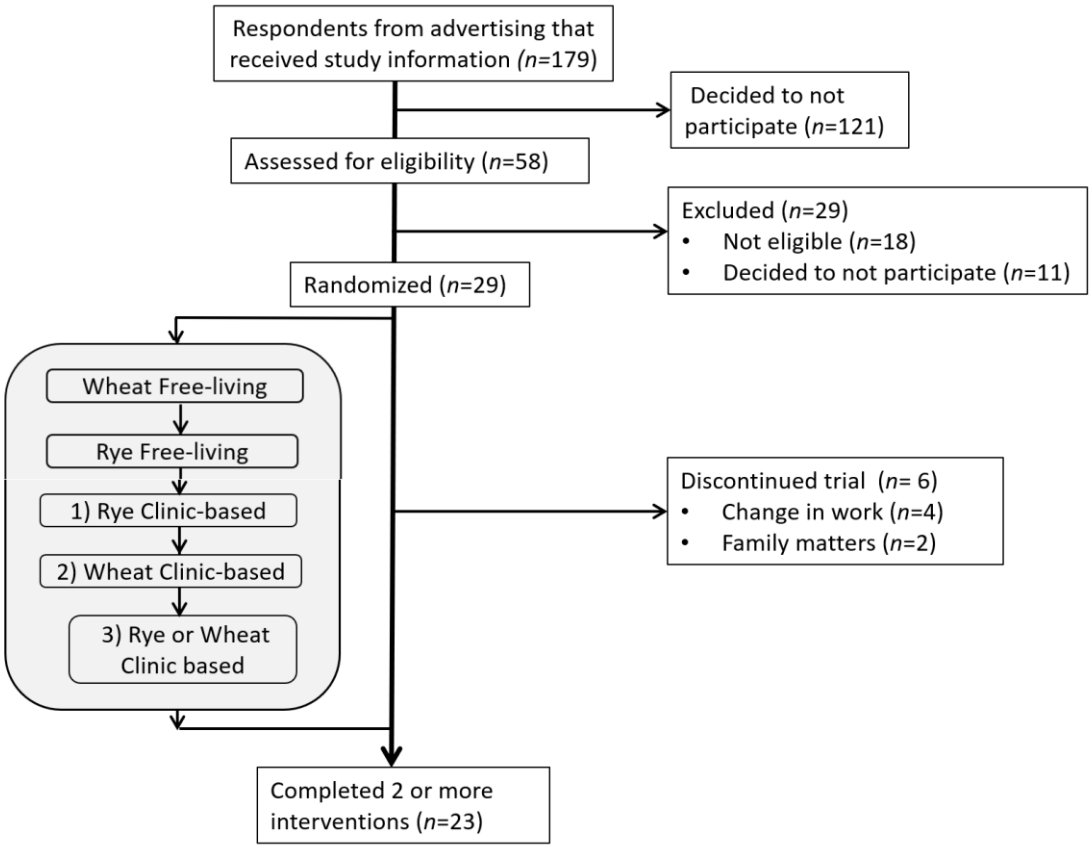

Figure 4.2.2.A. Flowchart of participants in the VASA-home trial (Papers II and III)

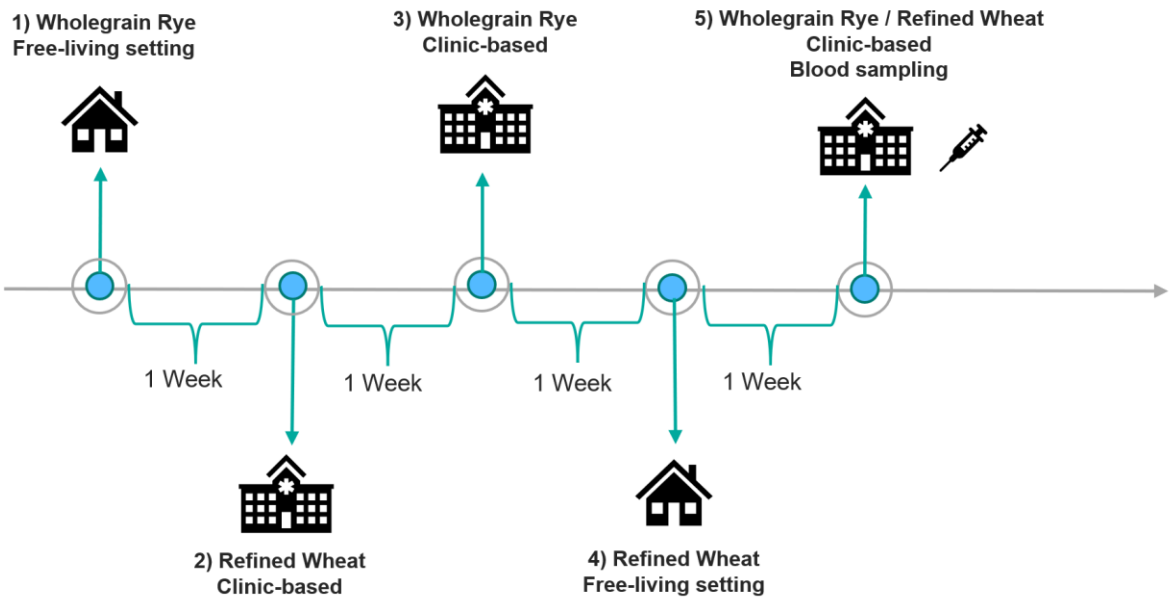

Figure 4.2.2.B. Overview of the study design and interventions in the VASA-home trial (Papers II and III)

Two interventions were performed in a free-living setting, two interventions in the research clinic and one additional intervention in the research clinic with continuous blood sampling where participants were randomized (50:50) to either rye or wheat-based diets. The last intervention day carried out in the research clinic with continuous blood sampling enabled analyses of incretins, ghrelin and inflammatory markers in response to the intervention diets. The sample size estimation was calculated with an alpha of 0.05 and power of 0.80 to detect 10% within-group differences in appetite ratings in different settings. Six-teen participants were required to complete interventions in both settings. However, to allow for secondary comparison of diets and maintain power to address planned exploratory analysis we aimed to randomize 30 participants.

Men and women 30-70 years of age, with a BMI of 27-35 kg/m2 were eligible for participation (for exclusion criteria see Paper II). Interstitial blood glucose was measured during all intervention days through CGM, while physical activity was monitored using pedometers. All foods were provided for both clinical-based interventions and intervention days in a free-living setting. Intervention foods were packed in neutral packaging and coded, but it is likely participants were aware of their allocation due to visual differences between rye and wheat products. Participants were randomly assigned to the intervention sequence using a computer-generated block randomization protocol. Each participant's assigned sequence was concealed in a numbered, opaque envelope, which was accessed sequentially after obtaining written consent.

4.2.3. The Ryeweight2 study

The RyeWeight2 study was conducted as a 12-week hypocaloric parallel randomized controlled trial in free-living participants (**Paper IV)**. The primary aim of the RyeWeight2 study was to investigate the effect wholegrain rye vs refined wheat foods within complex diets on body weight and body fat mass. The secondary aim was to assess the effects on metabolic risk markers, appetite, gut microbiota, and SCFAs, and their potential role in weight loss. The number of participants needed was estimated based on two primary endpoints (body weight and fat mass change), with power of 80% and alpha of 2.5%. To detect a 12-week intervention effect of 1 kg difference in body weight and 1% difference in body fat percentage 106 participants in each treatment group were required to complete the study. Based on experience from previous studies, we accounted for a drop-out rate of 18%, aiming to randomize 130 participants in each treatment group (**Figure 4.2.3**).

Men and women aged 30-70 years, with a BMI of 27-35 kg/m2, were eligible to participate in the study (for exclusion criteria see Paper II) and started with a 2-week run-in period following a hypocaloric refined wheat-based diet. Participants were then randomized to follow either a wholegrain rye or refined wheat-based diet for the following 12 weeks. At baseline week 6 and week 12, fecal samples were collected, and clinical examinations were undertaken with anthropometric measures, blood samples and body composition by dual energy X-ray absorptiometry (DEXA).

Intervention foods were packed in neutral packaging and coded, but it is likely participants were aware of their allocation due to visual differences between rye and wheat products. Study nurses as well as research staff were blinded. The study dietician was blinded when consulting participants in strategies to achieve daily calorie deficiency and include intervention foods into

habitual diets. Participants were randomly assigned to the intervention sequence using a computer-generated block randomization protocol. Each participant's assigned sequence was concealed in a numbered, opaque envelope, which was accessed sequentially after obtaining written consent.

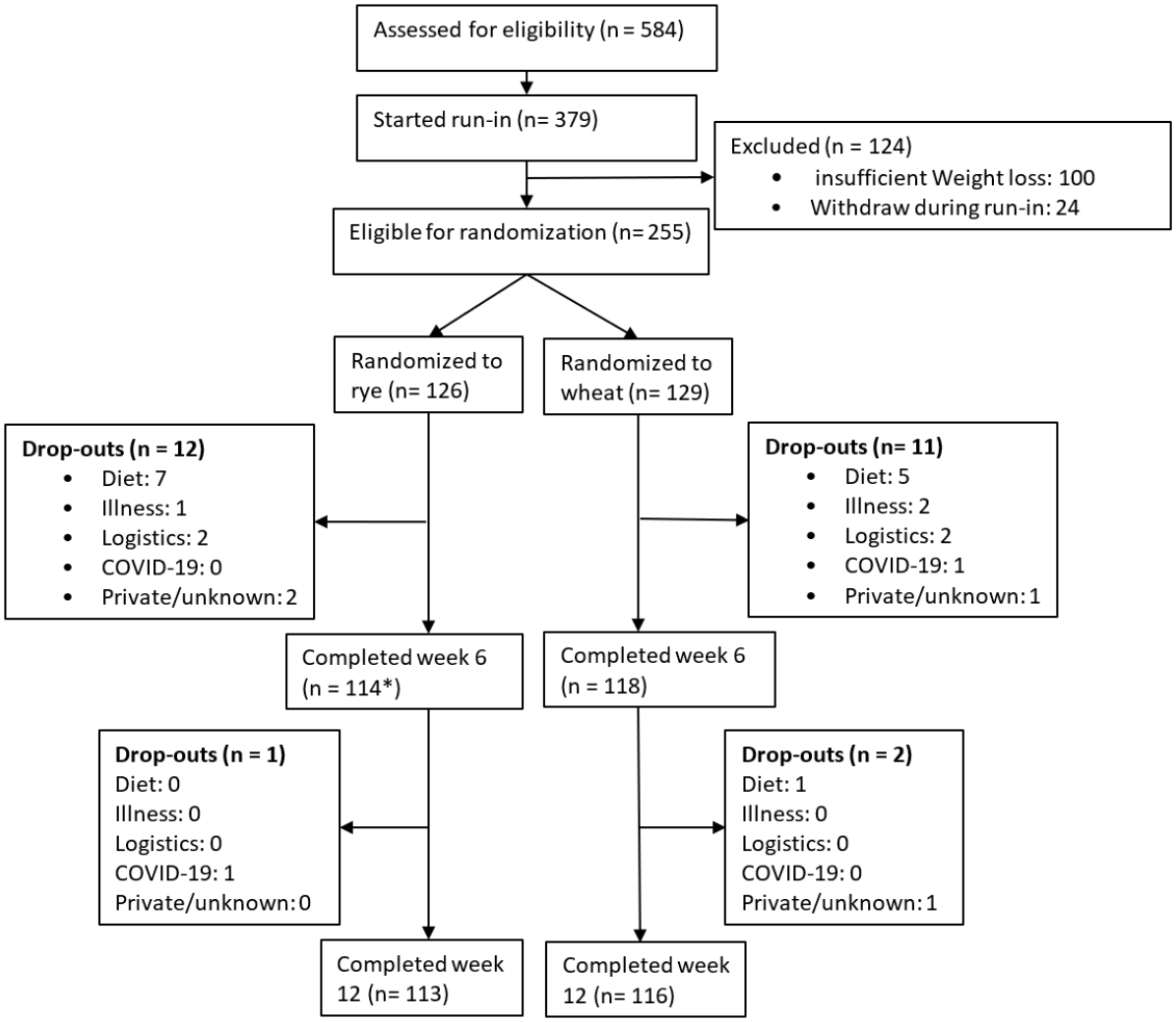

Figure 4.2.3. Flowchart of participants in the RyeWeight2 study (Paper IV). * Including 3 participants, not attending week 6 examination but completed the study and attended week 12 (defined as complete cases).

4.3. Intervention diets

4.3.1. The Whole-grain milling & glycemia trial

Habitual grain consumption was assessed at baseline by a 4-day semiquantitative food diary and participants' current grain intake was replaced by intervention foods for the two interventions. No advice was given to change the total amount of cereal consumed. Participants were not asked to restrict calories and no additional dietary or lifestyle advice was given. In one intervention, participants were provided with rolled oats, brown rice, and wholegrain bread made with coarsely ground flour and kibbled wheat kernels. In the other intervention, participants were provided with instant oats, brown rice pasta, and whole-grain bread made with finely milled wheat flour. There were differences between bread recipes in the amount of water used, however the amount of carbohydrate and fibre were matched per loaf and per slice. All intervention foods were 100% whole grains and were matched for macronutrients and fiber (**Table 4.3.1**). The intervention foods differed in the degree of milling as measured by particle size (Table 4.3.1). Participants were provided with checklists to record daily consumption of intervention foods and were instructed to return all remaining foods at the end of each intervention period for compliance evaluation.

Table 4.3.1. Nutritional composition of the intervention products used in the Whole-grain milling & glycemia trial (Paper I)

	Less-processed whole-grain intervention			Finely milled whole-grain intervention		
	Traditional oats (cooked)	Brown rice (cooked)	Coarsely milled bread	Instant oats (cooked)	Brown rice pasta (cooked)	Finely milled bread
Nutrients per 100 g						
Energy (kJ)	512	850	862	512	822	852
Carbohydrates (g)	18.4	39.5	35.77	18.4	40.5	36.49
Protein (g)	4.8	4.9	6.99	4.8	4.3	7.03
Fat (g)	2.3	2.2	2.45	2.3	1.6	1.79
Fiber (g)	4.0	1.6	9.2	4.0	1.4	9.1
Sodium (mg)	<5	<5	290	<5	6.5	290
Retention of whole grains on particle-size sieves (mm), %						
>2,800	93	0	23	40	0*	0
1,000–2,799	7	100	39	52	0*	0
180–999	0	0	16	4	6*	59
<180	0	0	22	4	94*	41

*These measurements were made on brown rice flour as the only listed ingredient in brown rice pasta.

4.3.2. The VASA-home trial

In the VASA-home trial intervention days were mimicking hypocaloric diets, hence individual energy requirements were estimated using equations developed by *Henry et al.* [136] and assuming a physical activity level (PAL) of 1.4. During all five intervention days, participants

followed an individual hypocaloric meal plan providing 1300–2300 kcal/day. Meal plans were calculated based on estimated energy requirements and a 500-kcal deficit. Participants consumed a fixed amount of either wholegrain rye (705 kcal) or refined wheat foods (685 kcal), providing approximately one third of the total energy intake for the average participant, other foods were adjusted to meet individual meal plans. Participants allocated to the wheat diet consumed 60g of extruded wheat puffs or semolina, 5 slices of crispbread, and a 72g baguette each day. Those allocated to the rye diet consumed 60g of extruded rye puffs or rolled rye flakes, 4 slices of crispbread and 117g of sliced rye bread each day. The amounts and nutrients provided from cereal foods throughout one intervention day are displayed in **Table 4.3.2**. (The energy content differs from published data (Paper II), as dietary fiber was not included in the energy calculation. The full-day meal plan included a breakfast consisting of cereal puffs with milk, a lunch with tomato and mozzarella soup, crisp bread and cheese/jam, an afternoon snack consisting of crisp bread with cheese/jam and a goulash soup with soft bread and jam/cheese for dinner. All food was provided for the study participants. Participants followed a strict menu with precise amounts of both foods and drinks and took notes in an attached checklist if any deviation occurred.

Table 4.3.2. Nutrients provided from rye and wheat intervention products at one intervention day in the RyeWeight 2 study (Paper IV) and VASA-home trial (Paper II and III).

	Product weight (g)	Energy (kcal)	CHO (g)	Protein (g)	Fat (g)	Dietary fiber (g)†		
						Total	*Extr.*	*Unextr.*
Extruded rye puffs	60	227	38.7	5.2	0.9	10.1	4.1	6.0
Crisp bread "Husman"	54	197	32.1	5.3	0.9	9.1	3.6	5.6
Soft rye bread	117	281	41.9	6.9	3.6	12.4	4.4	8.0
Total rye	*231*	*705*	*112.6*	*17.4*	*5.4*	*31.6*	*12.1*	*19.6*
Extruded wheat puffs	60	228	43.8	7.4	0.8	3.2	1.5	1.7
Wheat crispbread	66	260	42.6	8.1	4.4	3.7	1.0	2.7
Soft wheat bread	72	197	35.1	7.1	1.7	2.5	0.7	1.8
Total wheat	*198*	*685*	*121.5*	*22.6*	*6.9*	*9.5*	*3.3*	*6.2*

† Dietary fibre is contributing with 2.0 kcal/g as described by FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition 1997

4.3.3. The Ryeweight2 study

The RyeWeight2 study was a 12-week hypocaloric dietary intervention, and individual energy requirements were estimated using equations developed by *Henry et al.* [136] and assuming a physical activity level (PAL) of 1.4. Participants baseline habitual diet was assessed by a 3-day weighted food diary. This food diary was used as basis when study dieticians implemented the Step-wise Weight-determined Accumulative change Plan (SWAP) to achieve daily energy deficit of 500 kcal [137]. Participants were advised to introduce the four principles of the model one at a time. 1) Limit the intake of sweets, cakes and soft drinks 2) minimize the intake of fast-food 3) choose low fat and low sugar alternatives when grocery shopping 4) increase the intake of

vegetables (half the plate for lunch and dinner) 5) decrease portion sizes. Change in body weight decided when the individual was advised to take the next step in the SWAP model.

The dietician instructed participants to completely exclude cereals other than the provided intervention products. The cereal intervention foods used in the RyeWeight2 study were identical to those of the VASA-home trial (Table 4.3.2), although participants were able to choose from various options of rye crisp breads (**Table 4.3.3**). The rye foods provided approximately 30g of dietary fiber per day, compared to 8g from the wheat foods. Otherwise, the intervention foods were matched for energy and macronutrients. Participants were instructed to fill in a pre-coded compliance journal where they ticked off the fixed amount of intervention foods daily and recorded any deviations from the diet.

Table 4.3.3. Nutritional composition of the intervention products used in the RyeWeight 2 study (Paper IV) and VASA-home trial (Paper II and III).

	Product weight (g)	Energy (kcal)	CHO (g)	Protein (g)	Fat (g)	Dietary fiber (g)†		
						Total	*Extr.*	*Unextr.*
Extruded rye puffs	100.0	379	64.4	8.6	1.5	16.8	6.9	9.9
Rolled rye flakes	100.0	360	59.3	7.6	1.6	18.3	7.4	10.9
Rye crisp bread "Rågi" *	100.0	364	64.0	9.8	1.4	14.1	5.6	8.5
Rye crispbread "Delikatess" *	100.0	366	57.6	9.3	1.3	19.3	7.4	11.9
Rye crisp bread "Husman"	100.0	364	59.5	9.8	1.6	16.9	6.7	10.3
Rye crisp bread "Sport" *	100.0	368	60.9	9.4	1.3	17.5	6.1	11.4
Soft rye bread	100.0	240	35.8	5.9	3.1	10.6	4.4	8.0
Extruded wheat puffs	100.0	380	73.0	12.4	1.3	5.4	2.5	2.9
Wheat semolina	100.0	351	71.3	10	1	3	1.3	1.6
Wheat crispbread	100.0	394	64.5	12.2	6.7	5.6	1.5	4.0
Soft wheat bread	100.0	274	48.8	9.9	2.4	3.5	1.03	2.5

† Dietary fibre is contributing with 2.0 kcal/g as described by FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition 1997. * Varieties of rye crisp breads only selectable optional in the RyeWeight 2 study (Paper IV).

4.4. Outcome assessments

4.4.1. Clinical examinations

Participants in the Whole-grain milling & glycemia trial were assessed for eligibility at an initial screening visit. Baseline questionnaires were filled in and anthropometric measures taken at the research clinic and venous blood samples drawn by trained nurses at an adjacent outpatient clinic. Before and after each 2-week intervention, anthropometric measurements (height, weight, waist circumference and body composition) were recorded in duplicates and resting blood pressure measured three times. Body composition was measured by bioimpedance, and venous blood samples collected. Interstitial blood glucose was measured during both interventions

through CGM. The sensor was applied at the back of the non-dominant arm at the clinic and removed after completed interventions at the clinic by study personnel.

In the VASA-home trial, eligibility to participate was assessed at the research clinic. Height, weight and blood pressure were measured, and venous blood samples collected. Body weight was measured using a digital scale, followed by measurements of waist and hip circumferences, and sagittal abdominal diameter. All anthropometric measurements were taken twice, with participants dressed in underwear or light clothing. Blood pressure was measured in duplicates using an automated blood pressure monitor after the participant had rested in a supine position for 10 minutes.

Study participants wore CGM sensors during all five intervention days and sensors were applied onto the back of the participant's upper arm and removed by study personnel at the research clinic. Physical activity was monitored through pedometers, attached to a waistband at the hip by study personnel or self-administered during intervention days in free-living setting. One intervention day compromised continuous blood-sampling. An intravenous catheter was inserted into the upper arm to facilitate blood sample collection at 27 timepoints throughout the clinic-based intervention day. At the three clinic-based interventions, participants filled in questionnaires about physical activity and gastro-intestinal symptoms.

In the RyWeight2 study participants were screened for eligibility according to the same inclusion criteria and clinical assessments were identical to those in the VASA-home trial. The RyeWeight2 study comprised additional clinical study assessments at baseline (week 0), mid-intervention (week 6), and post-intervention (week 12). The three study assessments at the research clinic included measures of blood pressure, anthropometric measures and venous blood sampling. Additionally, participants underwent a full body DEXA scan for body composition assessment and filled in questionnaires about physical activity, gastro-intestinal symptoms and stool.

4.4.2. Dietary assessment

Habitual dietary intake was assessed in the Whole-grain milling & glycemia trial at baseline by 4-day semiquantitative food diaries. Food diaries provided data on participants' current grain intake, which was replaced by intervention foods in equal quantities for the two interventions. Participants also filled in 4-day semiquantitative food diaries during the finely milled and intact wholegrain intervention. These diaries were analyzed using software (FoodWorks 9, Xyris) and food databases *New Zealand FOOD files 2016* and *AusFoods 2017*. Alkylresorcinols (AR), were measured as an objective marker of whole grain intake trough liquid chromatography-high-resolution mass spectrometry [138].

No dietary assessment was conducted in VASA-home trail.

In the RyeWeight2 study, dietary intake was assessed at baseline, mid-intervention, and at the 12-week follow-up using a 3-day weighed food diary. Average daily energy and macronutrient intakes were calculated using software (Dietist Net Pro*)* and the *Swedish Food Composition Database* and intake of major food groups was assessed using the definitions provided by *Riksmaten* survey by the Sweden National Food Agency [139]. Plasma alkylresorcinols

concentrations were measured at Chalmers Mass Spectrometry Infrastructure using liquid chromatography-tandem mass spectrometry, following a method developed at the platform [140]. Total AR and a homologues C17:0-C25:0 reflected total whole grain intake from rye and wheat sources, while the AR C17:0/C21:0 ratio was calculated as marker of the proportion whole grain from wheat and rye sources respectively.

4.4.3. Subjective appetite assessment

In all three studies subjective appetite was assessed. The RyeWeight2 study and VASA-home trial utilized the same protocol for appetite assessment and results included in corresponding papers, while subjective appetite data from the Whole-grain milling & glycemia trial is published in a separate paper not included in this thesis [141].

In the Whole-grain milling & glycemia trial, appetite response was measured at three time points: at baseline and in the second week of both wholegrain interventions. On each occasion participants reported their perceived hunger and satiety on a 100m-VAS during 4 days. Participants reported their sensations of hunger before breakfast, lunch and dinner, while perceived satiety was reported within 10 minutes of completing the meals. All appetite assessments were performed in a free-living setting.

For the VASA-home trial and RyeWeight2 study foods and protocols for appetite assessments were identical. Day-long appetite assessments were conducted, and participants continuously answered questions about their appetite every 30 min from 8:00AM to 12:00PM and every 60 min from 12:00PM to 9:00PM (**Figure 4.4.3**). At each timepoint participants answered three questions in random order: “How hungry are you?”, “How full are you?”, “How big is your desire to eat? They marked their answer on a 100 mm VAS, anchored at each end with: “not hungry at all” and “very hungry”, “not full at all” and “very full”, “very weak” and “very strong”. Participants received an automated email through a software (Qualtrics, https://www.qualtrics.com), and completed the appetite questions online. An analogue option with questions on paper was also provided. A detailed schedule for the appetite assessment days was provided, outlining specific timepoints for appetite questions and meals.

The appetite assessment days were performed in a free-living setting and all foods were provided. These include wholegrain rye and refined wheat cereals, as well as milk for breakfast, tomato and mozzarella soup for lunch, cheese/jam for the snack, and goulash soup with cheese/jam for dinner. Participants followed an individual hypocaloric meal plan (4.3 Study diets) with a set menu for appetite assessment days. The cereal foods were either wholegrain rye or refined wheat according to allocation and the amount fixed, while other foods were adjusted to meet the individual meal plans. In the VASA-home trial three out of five interventions were clinic-based, hence the full appetite assessment was conducted at the research clinic for these interventions. This allowed for the main aim of the trial to evaluate the performance of VAS in measuring subjective appetite in a monitored clinical setting compared to under free-living conditions.

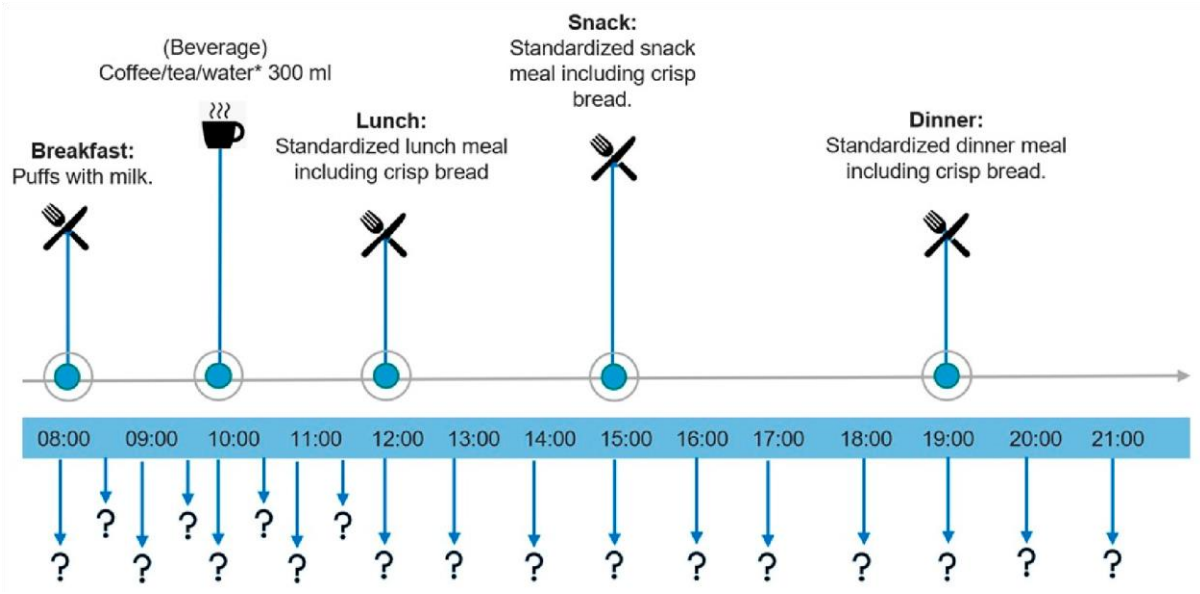

Figure 4.4.3. Intervention day overview with appetite questions and meal timings.

4.4.4. Clinical markers

In the Whole-grain milling & glycemia trial, blood samples were collected at screening to assess inclusion criteria and analyze Glycated hemoglobinA_{1c}(HbA_{1c}). Pre- and post-intervention fasting plasma samples were collected and stored at -80°C until analysis. Samples were analyzed for HbA_{1c}, cholesterol (total, LDL, and HDL), triglycerides and CRP on an automated analyzer (Roche Diagnostics). Fasting insulin was measured with a Bio-Plex magnetic bead array (BioRad Laboratories).

In the VASA-home trial, blood samples collected at screening were analyzed at Sahlgrenska University hospital and blood markers evaluated against inclusion criteria. During one out of five interventions, continuous blood samples were drawn. After insertion of an intravenous catheter in the morning, one fasting sample was collected, and then postprandial samples continuously collected the following 12 hours. A total of 27 timepoints throughout the intervention day. An inhibitor cocktail was added to tubes immediately after sampling to inhibit protease degradation of peptide hormones. Plasma samples were stored in -20 °C for a maximum of 7 days at the research clinic, before transferred to -80 °C awaiting analysis.

Blood samples collected during the screening process in the RyeWeight2 study were analyzed at the clinical chemistry laboratory at Sahlgrenska University hospital to evaluate blood markers for inclusion criteria. All blood samples collected at baseline, week 6 and week 12 were stored in a biobank at -80 °C awaiting analysis. Plasma samples were analyzed at Lycksele clinical chemistry laboratory, Umeå University Hospital, for glucose, CRP, insulin, cholesterol (total, LDL and HDL) and triglycerides. These metabolic markers were analyzed with Cobas® Pro (Roche Diagnostics, Basel, Switzerland) according to laboratory accredited methods.

In both the VASA-home trial and the RyeWeight2 study novel inflammatory markers glycoprotein N-acetylation (GlycA and GlycB) and supramolecular phospholipid composite peak (SPC) were

analyzed with proton nuclear magnetic resonance (NMR) spectrometry at Swedish Nuclear Magnetic Resonance Centre in Gothenburg.

4.4.5. Continuous glucose measures

Continuous glucose was measured in both the Whole-grain milling & glycemia trial and the VASA-home study. Both studies utilized the same CGM device (Abbott Freestyle Libre Pro IQ; Abbott Laboratories, Chicago, IL, USA) for Interstitial glucose measures. The CGM sensor is factory calibrated and glucose data is recorded every 15 minutes, stored in the sensor and blinded to participants. The sensor is waterproof, hence no restrictions to sanitation or recreational activities were needed. However, participants were instructed to avoid hot tubes, sauna or other conditions exposing the sensor for high temperature. In the Whole-grain milling & glycemia trial, CGM readings were obtained for the full duration of both 14-day interventions in participants under free-living circumstances. Participants wore CGM sensors for the three clinical based interventions, 8:00AM to 9:00PM in the VASA-home trial. In both studies CGM data was analyzed for postprandial glucose iAUC as well as measures of glycemic variability. Additionally, in the Whole-grain milling & glycemia trial, time in range, below and above range were considered as clinically relevant considering participants were diagnosed with type 2 diabetes.

4.4.6. Ghrelin and incretin hormones

In the VASA-home trial, a clinic-based intervention with continuous blood sampling was conducted. For each participant, 27 plasma samples were analyzed for a panel of hormones relevant in reflecting appetite regulating. Acyl-ghrelin, along with incretins GIP and active GLP-1, as well as total PYY were assayed by multiplex enzyme-linked immunosorbent assay (ELISA) using electrochemiluminescence detection. Samples were analyzed in duplicate on 96-well multispot plates (Meso Scale Diagnostics (MSD), Rockville, MD, USA) with specific capture antibodies and quantified using the MSD imager (QuickPlex SQ120). Intra-assay variability was obtained through a quality control sample on all plates and inter-assay variability calculated from duplicate samples. The PYY assay performed poorly and with over a third of the observations falling below the limit of detection and PYY data was subsequently excluded from analysis.

4.4.7. Inflammation markers measured by NMR spectroscopy

In the VASA-home trial, plasma samples from postprandial continuous sample collection were analyzed for novel inflammatory markers GlycA, GlycB and SPC. These inflammatory markers were also measured in the RyeWeight2 study in plasma samples collected at baseline and the 12-week follow-up. Samples from both studies were analyzed at the Swedish Nuclear Magnetic Resonance Centre, Gothenburg with proton nuclear magnetic resonance (NMR). NMR data was acquired at 310K on a Bruker 600 MHz Avance III HD spectrometer, and GlycA, GlycB and SPC composite inflammation markers were extracted with an in-house MatLab script.

4.4.8. Gut microbiota composition and SCFAs

In the RyeWeight2 study fecal samples were collected at baseline, week 6 and week 12 with fecal collection tubes (Sarstedt AG & Co., Germany) and EasySampler stool collection kit (GP Medical Devices ApS, Denmark), provided to participants. Participants were instructed to store fecal samples in a freezer (-18 ^{0}C) before delivery to the clinic, within 72 hours. Alternatively, samples were kept in provided cooling bags with frozen cooling blocks and delivered to the clinic within 24 hours. Fecal samples were stored in -20 ^{0}C for a maximum of 7 days at the research clinic, then transferred to -80 ^{0}C freezers for long-term storage.

Baseline fecal samples and samples collected at 12-week follow-up were analyzed for gut microbiota composition. DNA-extraction was performed using NucleoSpin Soil kit (Macherey-Nagel, 740780.250M) from 451 fecal samples and metagenomic shotgun sequencing performed in a MGIEasy Fast FS DNA Library Prep Set (MGI Tech Co., Ltd., 940-000030-00). A panel of 9 SCFA (formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, succinic acid, valeric acid, isovaleric acid and caprionic acid) were measured in baseline, 6-week and 12-week plasma samples at Chalmers University of Technology according to a recently developed method by *Fristedt et al*. [142].

4.4.9. Data analysis

Statistical analysis of the Whole-grain milling & glycemia trial data has been described in detail in Paper I. Briefly, CGM data and metabolic risk markers were analyzed according to intention to treat. A mixed model accounting for intervention order was built to analyze continuous glucose data. All statistical analysis was performed in Stata 15 (StataCorp, College Station, TX).

Subjective appetite data in the VASA-home trial (Paper II) were analyzed according to modified intention to treat, defined as participants completing two or more interventions were included in the final analysis. A linear mixed effects model was built to analyze mean appetite scores and evaluate the effect of location and diet. Intervention order was considered, and the model adjusted for baseline VAS-score, and subject fitted as random effects variable. Order of intervention was considered, and the model was adjusted for baseline VAS score, and subject was adjusted as the random effect variable. In addition, effects of continuous blood sampling were evaluated in all models and location and diet evaluated as covariates in their respective models. Appetite response was also evaluated as total area under the curve (tAUC). tAUC was calculated using approximating integrals according to the trapezoidal rule [143]. For tAUC analysis, no baseline covariate was fitted in the statistical model and imputation was performed using the mean values from corresponding timepoints on other intervention days “other day imputation” [144]. See Paper II for more details.

Data generated from continuous blood sampling and CGM in the VASA-home trial (Paper III), were analyzed according to complete case analysis. Complete cases were defined as participants completing both rye and wheat-based interventions at the research clinic. Midpoint carry-forward imputation was utilized for ghrelin and incretin data and tAUC calculated using approximate integrals. For CGM data, Random Forest (missForest, 1.4) imputation was applied and incremental AUC (iAUC) calculated.

Glucose iAUC was analyzed in a mixed effects model for repeated measures accounting for intervention order and continuous blood sampling. The model was adjusted for age, step count and subject fitted as random effects variable. Differences in gut hormone tAUC were evaluated in an analysis of covariance (ANCOVA) model adjusted for baseline concentrations and age. Mean concentrations of inflammation markers were evaluated using a baseline adjusted mixed model. All data in the VASA-home trial were analyzed using R (version 4.1.2) and data presented as estimated marginal means ± standard error of means (SEM). Probability (P) values reported underwent post-hoc Bonferroni correction for multiple comparisons adjustment.

In the RyeWeight2 study, all data were primarily analyzed according to complete case analysis, including participants that completed the final examination at week 12 and were considered compliant (consuming at least 80% of interventions foods). Primary outcomes body weight and fat mass were evaluated after 6- and 12-weeks using baseline-adjusted linear mixed-effects models with subjects included as a random effect variable unless otherwise specified. Given the two primary endpoints, Bonferroni adjustment was applied to account for multiple testing and $p < 0.025$, considered significant. Anthropometric measures and clinical markers were also evaluated in a baseline adjusted linear mixed-effects model and $p < 0.05$ was considered significant. Models were adjusted for body weight change when clinical markers were analyzed.

The effect of the diets on gut microbiota at species level was explored through machine learning modeling, using a random forest algorithm with unbiased variable selection. Additionally, baseline microbiota composition and associations with body weight and fat mass change were examined in a similar machine learning model and through linear modelling. Plasma SCFAs were evaluated at 6 and 12 weeks in a baseline adjusted linear mixed-effects model. SCFAs as determinants of body weight and fat mass change were considered in Spearman rank correlations and linear models. Subjective appetite data were analyzed as mean appetite scores and tAUC for postprandial periods and day-long response to intervention diets. Data were analyzed in mixed models and baseline adjusted for mean appetite scores.

5 RESULTS AND DISCUSSION

5.1. Participants, baseline characteristics, dietary intake and compliance

Table 5.1. Baseline characteristics of all study participants across the three studies.

	the Whole-grain milling & glycemia trial (Paper I)	the VASA-home study (Paper II & III)	the RyeWeight2 study (Paper IV)
Number of participants	31	29	229
Number of women (%)	14 (45)	21 (72)	153 (67)
Age (years)	63.2 ± 12.7	56 ± 13	55 ± 10
Weight (kg)	92.7 ± 21	87 ± 13	87 ± 11
BMI (kg/m^2)	32.5 ± 6.8	32 ± 9	29.6 ± 2.3
Systolic BP (mmHg)	130 ± 17	130 ± 13	124 ± 12.5
Alkylresorcinols (nmol/L)	47.7 ± 39.2	NA	31.5 ± 12.5

Data are presented as means ± SD. Abbreviations: BMI, body mass index; BP, blood pressure.

5.1.1. The Whole-grain milling & glycemia trial

The Whole-grain milling & glycemia trail was conducted in Dunedin, New Zealand and participants with type 2 diabetes were recruited trough advertisements in social media, fliers in supermarkets and general practices. Nearly 90% of participants in the trial self-identified as being of European ethnicity. The distribution between men and women was relatively even (**Table 5.1**). The average duration of diabetes among participants was 11.4 ± 9.1 years and most participants were on oral hypoglycemic agents.

Thirty-one participants commenced the trial and 28 (90%) completed both interventions. Two participants were excluded after randomization upon discovering they did not meet eligibility criteria, and one participant passed away from causes unrelated to the study.

Participants increased their total energy intake from carbohydrates and decreased intake from fat slightly, comparing food diaries from baseline and during wholegrain interventions. Dietary fibre intake increased from 25.8 ± 9 to 40 ± 11 and 38.3 ± 11 for the intact grains and finely milled grain intervention respectively. Intakes of all macronutrients and fiber were similar during the two wholegrain interventions. Total AR as objective measure of compliance increased with similar magnitude during both interventions and were approximately three times higher compared to baseline. Participants reported daily intake of 5.5 servings of wholegrain intervention foods. No difference was observed between the interventions, as expected, since participants' usual grain

consumption—assessed by baseline food diaries—determined the whole grain amounts for both interventions.

5.1.2. The VASA-home trial

The VASA-home trial was conducted in Gothenburg and participants recruited through advertisements in social media, local newspapers in Gothenburg and surrounding areas, and through registrations on the online recruiting website (www.accindi.se). Twenty-nine participants were randomized which of 90% stated Sweden as their country of birth. Twenty-three participants (79%) completed two or more out of the five interventions, and six participants discontinued the trial, due to changes in working hours (*n* = 4) and family matters (*n* = 2). Twenty-one study participants (72%) completed the third clinic-based intervention with continuous blood sampling (**Table 5.1.2**). As these participants randomized 50:50 to wheat and rye-based diets for this specific intervention day baseline characteristics needs to be considered for groups separately. Participants in the rye-group were slightly older, approximately 6kg heavier and considerably higher HOMA-IR (Table 5.1.3). In a small study population like this, randomization into two groups carries the risk of skewed distribution of anthropometric and metabolic characteristics between the groups. The differences in baseline metabolic status between groups need to be considered when interpreting results.

During interventions-days in free-living setting participants reported adherence to the individual hypocaloric meal plan through checklists provided. All participants were considered compliant except for one individual who was excluded from analysis in Paper III due to substantial deviations from the study diet.

Table 5.1.2. Baseline characteristics of participants that completed the third intervention in the VASA-home trial (Paper III).

	The VASA-home trial Intervention 3	
	Rye-group *n = 9*	Wheat-group *n = 11*
Female (%)	67%	73%
Age	58.9 ± 10.5	56.5 ± 12.6
Weight	92.0 ± 18.0	85.7 ± 9.5
BMI	30 ± 2.9.0	30.1 ± 1.9
Systolic B.P	132.8 ± 10.2	131.5 ± 12.6
HOMA-IR*	4.7 ± 6.3	1.9 ± 1.0

Data are presented as means ± SD. Abbreviations: BMI, body mass index; BP, blood pressure; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance. *Outlier identified in the rye-group.

5.1.3. The Ryeweight2 study

The RyeWeight2 study was conducted in Gothenburg, Sweden and adults with overweight or obesity recruited through advertisements in newspapers, on social media and through registrations on the online recruiting website (www.accindi.se). The majority of participants (83%) stated Sweden as their country of birth and education level, annual income and current occupation were similar between participants randomized to the rye and wheat-group. The aim was to recruit as many men as women, but two thirds of participants completing the 12-week interventions were female and the sex distribution was thus slightly skewed. More details are provided in Paper IV. In total 255 participants were randomized and 229 (90%) completed the 12-week intervention (Figure 4.2.3).

Sixteen participants discontinued the intervention, due to gastrointestinal problems or disliking the diet (n = 13), being sick (n = 5), logistic reasons (n = 4) and private reasons (n = 4). Details on drop-outs due to COVID-19 are provided in Paper IV. The baseline characteristics for all participants completing the RyeWeight2 study are presented in Table 5.1, and baseline characteristics by the wheat (n = 116) and rye-group (n = 113) respectively are presented in **Table 5.1.3**. No significant differences in baseline characteristics were observed between the rye- and wheat-group in body weight, BMI, systolic or diastolic blood pressure, or plasma alkylresorcinols.

Table 5.1.3. Baseline characteristics of study participants by diet-group in the RyWeight2 study (Paper IV).

	the RyeWeight2 study (Paper IV)	
	Rye-group n = 113	Wheat-group n = 116
Number of women (%)	72 (64%)	81 (70%)
Age (years)	56.1 ± 9.6	54 ± 10.3
Weight (kg)	86.9 ± 10.4	87.1 ± 11.7
BMI (kg/m^2)	29.7 ± 2.1	29.5 ± 2.4
Systolic BP (mmHg)	123.8 ± 12	124.6 ± 13
Alkylresorcinols (nmol/L)	33.6 ± 27	29.6 ± 14

Data are presented as means ± SD. Abbreviations: BMI, body mass index; BP, blood pressure

Both intervention groups reduced their total energy intake with 200-250 kcal/day, while protein intake was slightly increased and energy from fat reduced with approximately 250 kcal/day in both intervention groups. A difference in dietary fiber intake was observed between the groups: the wheat-group reported 19g/day, while the rye-group reported 42g/day, reflecting the higher fiber content of the rye-based intervention products. We also noted a slightly higher protein intake among participants in the wheat-group. Intake of dairy foods (excluding cheese) were similar at baseline, approximately 250 g/day and increased to 350 g/day g/day in the rye-group and almost

400 g/day in the wheat-group. This small, yet significant difference may explain observed differences in protein intake. Participants in the wheat-group consumed 63% net weight cereal puffs for breakfast, compared to 51% net weight cereal puffs for breakfast in the rye-group. Participants in the rye-group could choose from four different varieties of crispbreads, with no single variety accounting for more than a third of the group's total crispbread consumption.

All participants who completed the rye and wheat interventions were considered compliant (> 80% of intervention foods consumed) according to compliance journals. Plasma concentrations of AR also indicated an overall good compliance (**Figure 5.1.3**). The groups had similar C17:0/C21:0 ratio and total AR at baseline, as expected after 2 weeks of run-in with wheat foods. After 6 weeks of intervention, average total AR concentrations in the rye-group was 10 times higher compared with concentrations in the wheat-group and still. At week 12, total AR was 7 times higher in the rye-group, and the difference in C17:0/C21:0 ratio remained high.

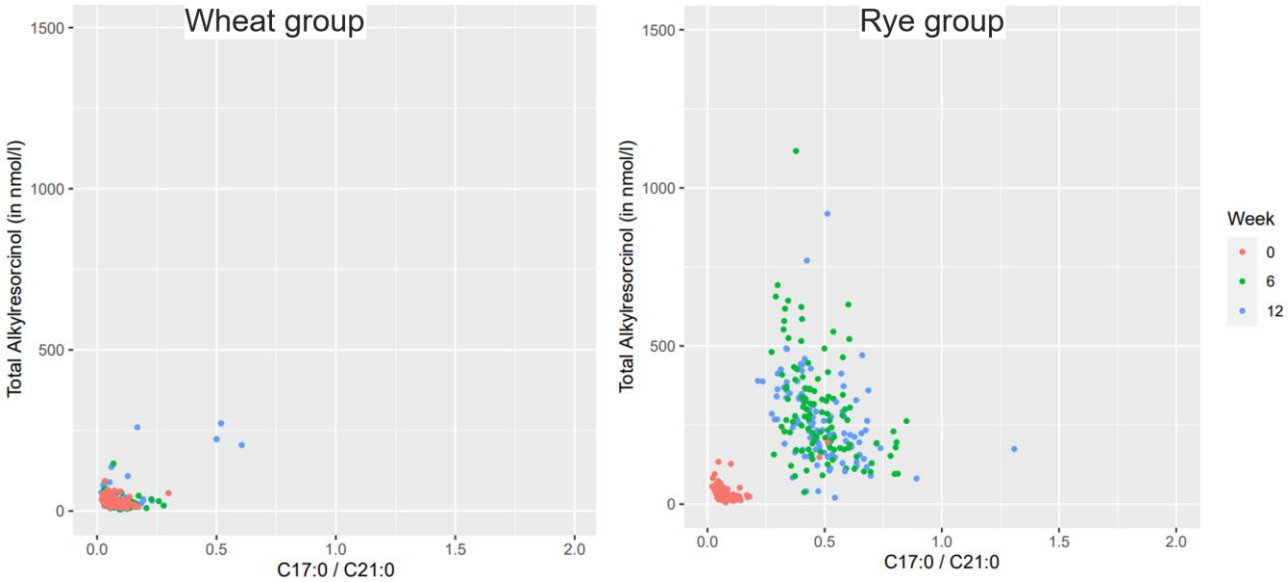

Figure 5.1.3. Total plasma alkylresorcinols and the C17:0/C21:0 ratio in the RyeWeight2 study. Baseline / Week 0 = red, week 6 = green, week 12 = blue.

5.2. Effects of whole grain interventions on glycemic control

5.2.1. Whole grain particle size

In the Whole-grain milling & glycemia trial, the primary outcome measure was glucose iAUC following breakfast, lunch and dinner for the two 14-day wholegrain interventions. The 180min postprandial iAUC following breakfast meals with intact vs finely milled wholegrain foods was 9% lower an average over the 2-week intervention period (**Table 5.2.1**). The breakfast, lunch and dinner 180min average postprandial iAUC showed a similar trend with 6% lower glucose iAUC following meals with intact wholegrain foods.

Whole grains have shown to improve postprandial glycemia compared to refined alternatives [20,31,55,56]. However, few studies have investigated the effect of wholegrain processing, specifically wholegrain particle size on glycemic response [35,145–147]. The longest and most similar intervention by *Järvi et al*. [35] investigated two nutrient-matched diets differing in glycemic index. Contrasts in glycemic index were achieved by including starchy foods with different particle size, including a wholegrain barley bread made of finely milling flour or whole barley seeds. When day-long glycemia was assessed, in the research clinic at the end of each intervention, glucose iAUC was improved for low glycemic index, larger-particle foods compared with the high GI, small particle size foods [35]. In contrast to *Järvi et al*. we measure glycemia in a free-living setting where participants selected from a variety of wholegrain foods to incorporate into their usual diet, increasing generalizability of results. To our knowledge, our trial is the first wholegrain intervention to evaluate day-long glycemic variability with CGM. We observed reduced glycemic variability measured as SD of daily glucose mean and MAGE, when participants consumed intact vs finely milled whole grains (**Table 5.2.1**). This improvement in glycemic variability trough diet, might be particularly relevant in patients with moderate diabetes, seeking to control their postprandial glucose [148].

In summary, intact whole grains, compared to finely milled whole grains, significantly reduced postprandial glucose levels and glycemic variability, highlighting the impact of whole grain particle size in glycemic control.

Table 5.2.1. Measures of glycemia calculated from CGM (Paper I).

Measure	Less-processed whole grains	Finely milled whole grains	P value difference between interventions
Meal responses (mmol/L/min)			
All-meal iAUC	423 ± 210	466 ± 192	0.022*
Breakfast iAUC	449 ± 256	525 ± 248	0.007*
Lunch iAUC	412 ± 287	440 ± 304	0.614
Dinner iAUC	391 ± 293	415 ± 277	0.117
Measures of glycemic variability			
MAGE	5.61 ± 2.75	5.94 ± 2.60	0.014*
CONGA	8.07 ± 2.49	8.20 ± 2.85	0.496
SD of daily mean (mmol/L)	2.33 ± 1.07	2.51 ± 1.10	0.002*

Data are mean ± SD. All values have been log-transformed to address skew. CONGA, continuous overall improvement in net glycemic action; MAGE, Mean Amplitude of Glycemic Excursion. Significant difference between diets in the same postprandial period is indicated by *($p < 0.05$).

5.2.2. Wholegrain rye vs refined wheat

In the VASA-home trial interstitial glucose was measured from 08:00AM in the morning to 9:00PM in the evening, during three clinic-based intervention days. Day-long glucose iAUC was reduced by 30% following the wholegrain rye vs refined wheat-based diet (**Table 5.2.2**). The improved

glycemic response was more pronounced in the morning and early afternoon. Also, in this study we investigated measures of glycemic variability, and observed lower SD of glucose mean for rye-based intervention days. Additionally, we calculated postprandial peak glucose concentration (Cmax). The Cmax was lower following the rye-based breakfast and snack meal as can be seen in (**Figure 5.2.2.**) showing the mean glucose curve for rye and wheat-based intervention days.

Table 5.2.2. Measures of glycemia calculated from CGM (Paper III).

Measure	Wholegrain Rye	Refined Wheat	P value difference between interventions
Meal responses (mmol/L/min)			
Breakfast iAUC	143 ± 21.7	198 ± 22.5	<0.0001**
Lunch iAUC	147 ± 22.3	198 ± 25.0	0.019*
Snack	73.9 ± 13.4	74.5 ± 16.1	0.97
Dinner iAUC	245 ± 22.1	285 ± 25.2	0.084
Measures of glycemic variability			
CV	18.5 ± 1.24	20.0 ± 1.34	0.13
SD of daily mean (mmol/L)	1.08 ± 0.09	1.21 ± 0.09	0.04*
MAGE	0.33 ± 0.03	0.38 ± 0.03	0.06

Data are presented as estimated marginal means ± SEM, *n = 21*. Significant difference between diets in the same postprandial period is indicated by *($p < 0.05$) and **($p < 0.005$). CV, Coefficient of Variation and MAGE, Mean Amplitude of Glycemic Excursion.

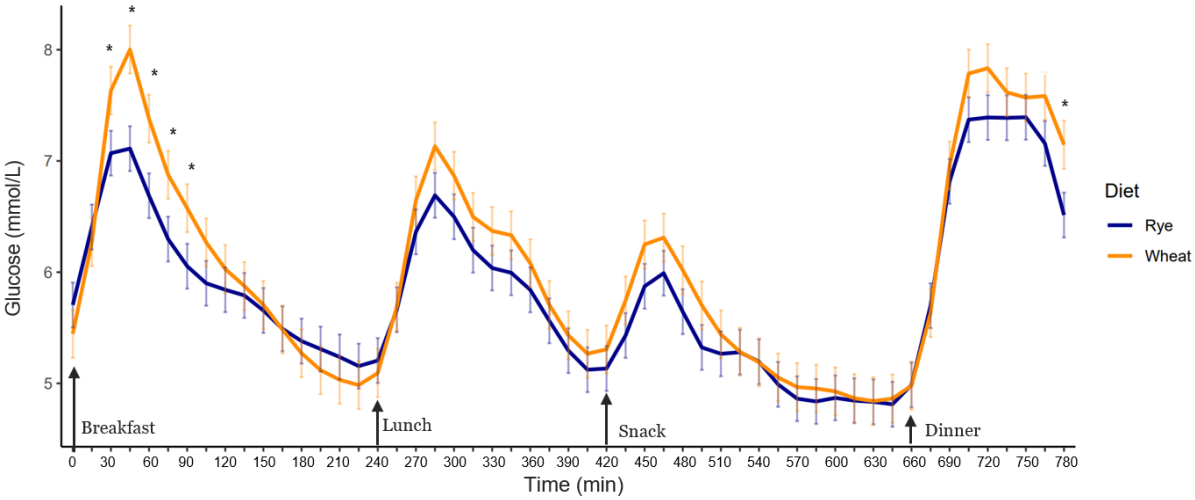

Figure 5.2.2. Mean glucose concentrations over the whole day 0-780min following wholegrain rye and refined wheat-based interventions. Significant difference between diets at certain timepoints are indicated by *($p < 0.05$). Data are presented as estimated marginal means ± SEM, n = 21

Few studies have investigated short- and long-term glycemic response following wholegrain rye interventions. However, a recent narrative review on this topic [31], identified three studies by *Rósen et al*. [24,62,63] and two more studies [21,23] showing reduced postprandial glucose AUC. These were all acute meal interventions, assessing postprandial responses to meals composed solely of wholegrain rye versus refined wheat foods. Additionally, several studies have demonstrated effects on postprandial insulin, without effects on glucose response, a phenomenon referred to as the "rye factor" [31]. Some studies have investigated effects of replacing habitual cereals with wholegrain rye [31], and only one intervention by *Mcintosh et al. 2003* showed reduced 1-hour postprandial glucose concentrations during a post-intervention test meal comparing high-fiber rye to low-fiber refined wheat [102]. Our study is the first to demonstrate improved day-long glycemia with wholegrain rye vs refined wheat foods as part of complex meals. Moreover, we demonstrated effects of wholegrain rye foods on glycemic control in individuals with overweight and obesity, previously only shown after meals composed entirely of rye foods [21].

Recently, a study investigating *in vitro* simulated gastrointestinal digestion of wheat and rye products included in this study and showed higher glucose and maltose release during digestion of the refined wheat products [61]. This was parallelled with measured higher viscosity of soluble arabinoxylans in the rye versus wheat foods, which may slow gastric emptying and limiting glucose absorption rate in the small intestine. This is possible mechanistic explanations for observed effects of rye foods on postprandial glycemic control.

In summary, replacing refined wheat with wholegrain rye foods in complex meals reduced glucose iAUC and measures of glycemic variability throughout the day, indicating improved glycemic control in individuals with overweight or obesity.

5.3. Subjective appetite in free-living vs-controlled setting

In the VASA-home trial (Paper II) we examined the performance of VAS under free-living conditions compared with traditional monitored clinical conditions. Overall, self-reported appetite in a clinical setting compared to under free-living conditions were similar. Fullness measured as tAUC was slightly higher (7.1 %, $p < 0.008$) in the clinical setting compared to free-living setting, driven by higher reported fullness following the afternoon snack (**Figure 5.3**).

To our knowledge, studies aiming to evaluate VAS in assessment of appetite response to diets are scarce. Recently, digital VAS were showing comparable results following a breakfast comprised of wholegrain cereals "at home" and in traditional laboratory conditions [149]. In contrast to our trial, the participants were not overweight (BMI <25), and VAS evaluated for a single meal response. It has been suggested that individuals with overweight and obesity have impaired appetite signaling and interventions focusing on this group may therefore be of relevance [25].

In summary, contrary to our hypothesis, results from self-reported appetite measurements were similar under both free-living and controlled clinical condition, suggesting this method can be used for evaluation of appetite response between diets under free-living conditions.

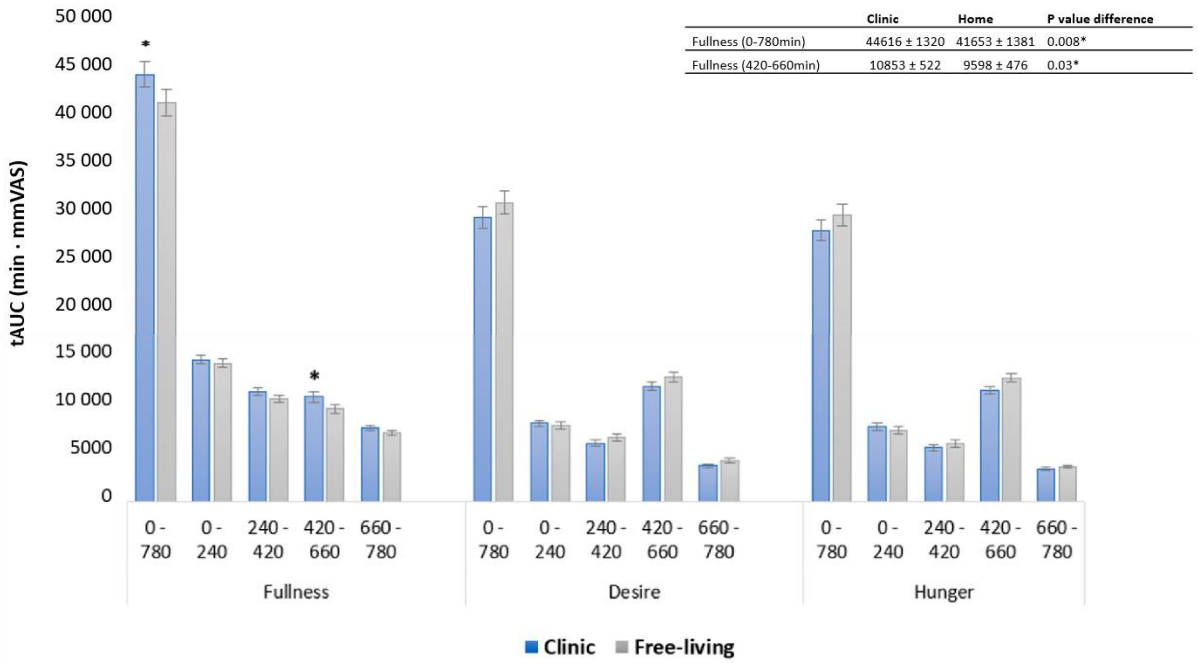

Figure 5.3. Subjective rating of fullness, hunger and desire to eat, measured as tAUC for free-living compared with clinical setting in postprandial periods: whole day 0–780 min, breakfast 0–240 min, lunch 240–420 min, snack 420–660, dinner 660–780 min. Significant difference ($p < 0.05$) between locations in the same postprandial period is indicated by *. Data are presented as least square means ± SEM, $n = 23$.

5.4. Subjective appetite and wholegrain rye

Subjective appetite response to wholegrain rye vs refined wheat-based diets were assessed in both the VASA-home trial and the RyeWeight2 study (Paper IV). The study diets and protocol for intervention days to evaluate differences in subjective appetite were identical between the two studies.

Overall, there were small differences in self-reported appetite between diets in the VASA-home trial. No differences in reported hunger, fullness, or desire to eat were observed, when whole-day responses were considered (8:00AM to 9:00PM). Although, following rye-based dinners, participants reported 12% ($p < 0.016$) higher fullness, and 17% lower hunger ($p < 0.02$) compared to wheat-based dinners (**Figure 5.4.C**). Analysis of mean VAS-scores confirmed satiating effects of the rye-based dinner and showed 15% lower hunger ($p < 0.05$) as well as 20% lower ($p < 0.04$) desire to eat following the rye vs wheat-based lunch.

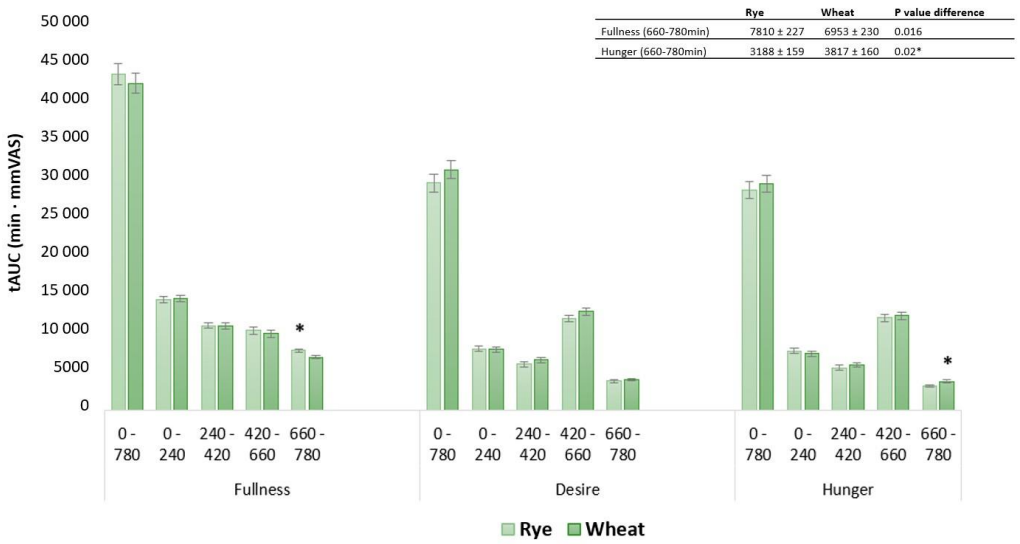

Figure 5.4.C. Subjective rating of fullness, hunger and desire to eat, measured as tAUC for rye- and wheat-based diets in postprandial periods: whole day 0–780 min, breakfast 0–240 min, lunch 240–420 min, snack 420–660, dinner 660–780 min. Significant difference ($p < 0.05$) between diets in the same postprandial period is indicated by *. Data are presented as least square means ± SEM, $n = 23$.

In the RyeWeight2 study, no differences in hunger, fullness, or desire to eat, measured as tAUC, were observed between diets at any of the three appetite assessment occasions (week 0, week 6, and week 12) (**Figure 5.4.A**). Mean VAS-scores for some specific timepoints throughout the appetite assessment day were different between the two diets (**Figure 5.4.B)**. At the 6-week assessment, the wheat-group reported lower hunger at 10:30 and lower desire to eat 11:00. At the 12-week assessment the wheat-group reported lower desire to eat at 8:30, while the rye-group reported higher fullness at 21:00. No associations between subjective appetite response and changes in body weight and body fat were observed. Energy intake and body composition have been closely tied to appetite regulation [8], but no such relationship could be demonstrated in the current study, nor was it found in the RyeWeight1 study [37].

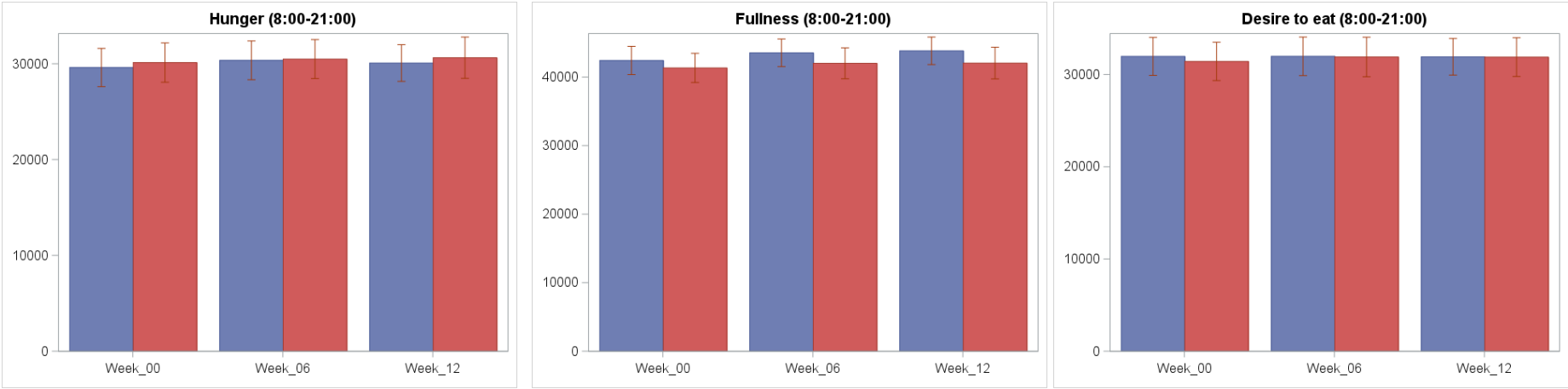

Figure 5.4.A. Total area under the curve (tAUC) of appetite responses at week 0, week 6 and week 12 measured over the whole day (8:00-21:00). The rye-group is illustrated in blue and the wheat-group in red. There was no significant difference between the groups at any timepoint (p>0.1).

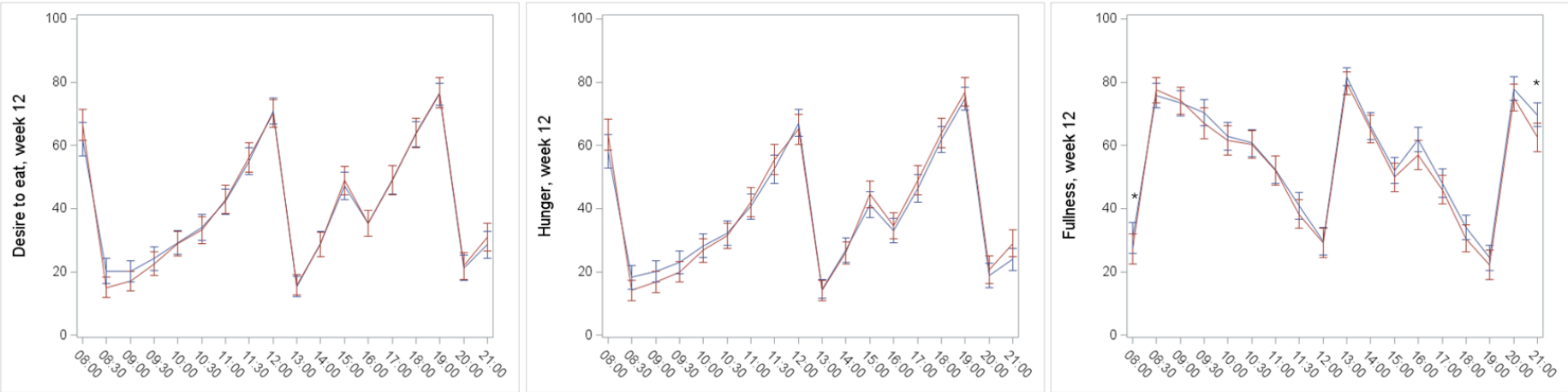

Figure 5.4.B. Appetite response measured as mean VAS-scores for the rye-group (illustrated in blue) and wheat-group (illustrated in red) at week 12. Significant differences ($p < 0.05$) between diets are indicated by: *. Data are presented as mean VAS-scores and 95% CI. Complete cases: rye-group (n=113), wheat-group (n=116).

Several trials have demonstrated increased satiety after consumption of wholegrain rye foods compared with refined cereals as acute meal responses [21–24,79]. In only one of these trials in participants with overweight or obesity, a breakfast composed entirely of rye, reduced hunger, while no effect on satiety or prospective consumption was observed [21]. The reduced hunger following the rye porridge breakfast extended into the postprandial period after a standardized lunch, with the effect becoming even more pronounced after lunch. Our results from the VASA-home trial align with those of *Hartvigsen et al.* [21] in similar participants (BMI 27–35), showing a similar pattern, where rye foods seem to promote satiety later in the day. However, in the VASA-home trial cereal intervention foods provided approximately one-third of total meal calories. Refined grains were substituted with wholegrain foods within the context of a complex diet, increasing the generalizability of these results. This is a strength of the trial, but it may also explain why our results differ from previous short-term studies, testing meals consisting solely of rye cereals.

As mentioned above, subjective appetite response to identical wholegrain rye and refined wheat foods in the RyWeigh2 study did not differ. This aligns with findings from the RyeWeight1 study, where, despite some differences in appetite responses at specific time points, the overall results showed no significant differences between the rye and wheat-based meals. This discrepancy between studies may be attributed to differences in clinic-based versus free-living settings, though our evaluation in the VASA-home trial showed that results were consistent across both settings. However, it cannot be ruled out that participants in the free-living setting could be influenced by environmental cues including aromas, talk about food and visual impressions that induce feelings of hunger and desire to eat [73,78]. These factors could theoretically induce noise and make it harder to identify contrast in appetite response between diets in the free-living setting. Additionally, large inter-individual variability has been reported in studies assessing subjective appetite, advocating for crossover study designs [150,151]. Nevertheless, indications of reduced hunger and increased fullness after consecutive wholegrain rye-based vs. refined wheat-based meals observed in the VASA-home trial should be interpreted with caution.

In summary, no differences in whole day appetite response were observed between the rye- and wheat-based diet in any of the studies. However, in the VASA-home trial in a tightly controlled clinical setting, rye-based meals induced higher fullness and lower hunger compared to wheat-based meals, particularly after dinner.

5.5. Appetite-regulating hormones and wholegrain rye

In the VASA-home trial a panel of gut hormones were analyzed as objective markers of appetite in parallel with subjective reporting of appetite response to wholegrain rye and refined wheat-based diets. There were no differences in GIP, ghrelin or GLP-1 tAUC measured over the whole intervention day (**Table 5.5.A**). However, GIP tAUC following the rye vs wheat-based lunch was 31% ($p < 0.05$) lower and mean ghrelin concentrations were 29% ($p < 0.05$) lower after the rye vs wheat-based dinner. Significant timepoints driving contrasts in postprandial periods are shown in **Figure 5.5.A**.

Table 5.5.A. Postprandial gut hormone response

Measure	Wholegrain Rye	Refined Wheat	P-value difference between diets
Whole-day GLP-1	6146 ± 1726 **(9138 ± 1944)**	8181 ± 1273 **(15284 ± 1757)**	0.37 **(0.03)**
Breakfast GLP-1	1723 ± 597 (2596 ± 601)	2674 ± 440 (4311 ± 543)	0.24 (0.05)
Lunch GLP-1	1657 ± 438 **(2231 ± 513)**	2280 ± 323 **(4312 ± 463)**	0.29 **(0.01)**
Snack GLP-1	1553 ± 405 **(2651 ± 515)**	1937 ± 299 **(4255 ± 466)**	0.47 **(0.04)**
Dinner GLP-1	1213 ± 399 (1640 ± 395)	1290 ± 294 (2638 ± 357)	0.88 (0.08)
Whole-day GIP	20915 ± 2698 **(1732 ± 733)**	26205 ± 2438 **(4449 ± 540)**	0.17 **(0.02)**
Breakfast GIP	5633 ± 643 **(480 ± 288)**	7251 ± 581 **(1440 ± 212)**	0.08 **(0.03)**
Lunch GIP	5168 ± 800 **(469 ± 210)**	7497 ± 723 **(1277 ± 155)**	**0.047** **(0.01)**
Snack GIP	6301 ± 909 **(439 ± 173)**	7402 ± 822 **(1048 ± 127)**	0.38 **(0.02)**
Dinner GIP	3774 ± 579 (343 ± 169)	4515 ± 523 (684 ± 125)	0.36 (0.14)
Whole-day Ghrelin	159514 ± 12185 (76329 ± 13562)	174014 ± 10991 (105936 ± 12233)	0.40 (0.13)
Breakfast Ghrelin	49884 ± 2816 (24698 ± 3584)	47342 ± 2540 (28573 ± 3233)	0.52 (0.44)
Lunch Ghrelin	38189 ± 3902 (18512 ± 3856)	42586 ± 3520 (26063 ± 3478)	0.42 (0.17)
Snack Ghrelin	52812 ± 4816 (24611 ± 4622)	59990 ± 4344 (36564 ± 4169)	0.29 (0.08)
Dinner Ghrelin	18629 ± 2148 **(8507 ± 1998)**	24096 ± 1937 **(12735 ± 1802)**	0.08 **(0.04)**

Postprandial gut hormone responses: glucose-dependent insulinotropic peptide, (GIP), ghrelin and glucagon-like peptide-1 (GLP-1) measured as tAUC for rye- and wheat- based diets in postprandial periods: whole day 0–755 min, breakfast 0–230 min, lunch 240–410 min, snack 420–650, dinner 660–755 min. Significant differences between diets ($p < 0.05$) are highlighted in bold. Data are presented as estimated marginal means ± SEM and in parentheses (estimated marginal means ± SEM in model normalized to baseline HOMA-IR), n = 20.

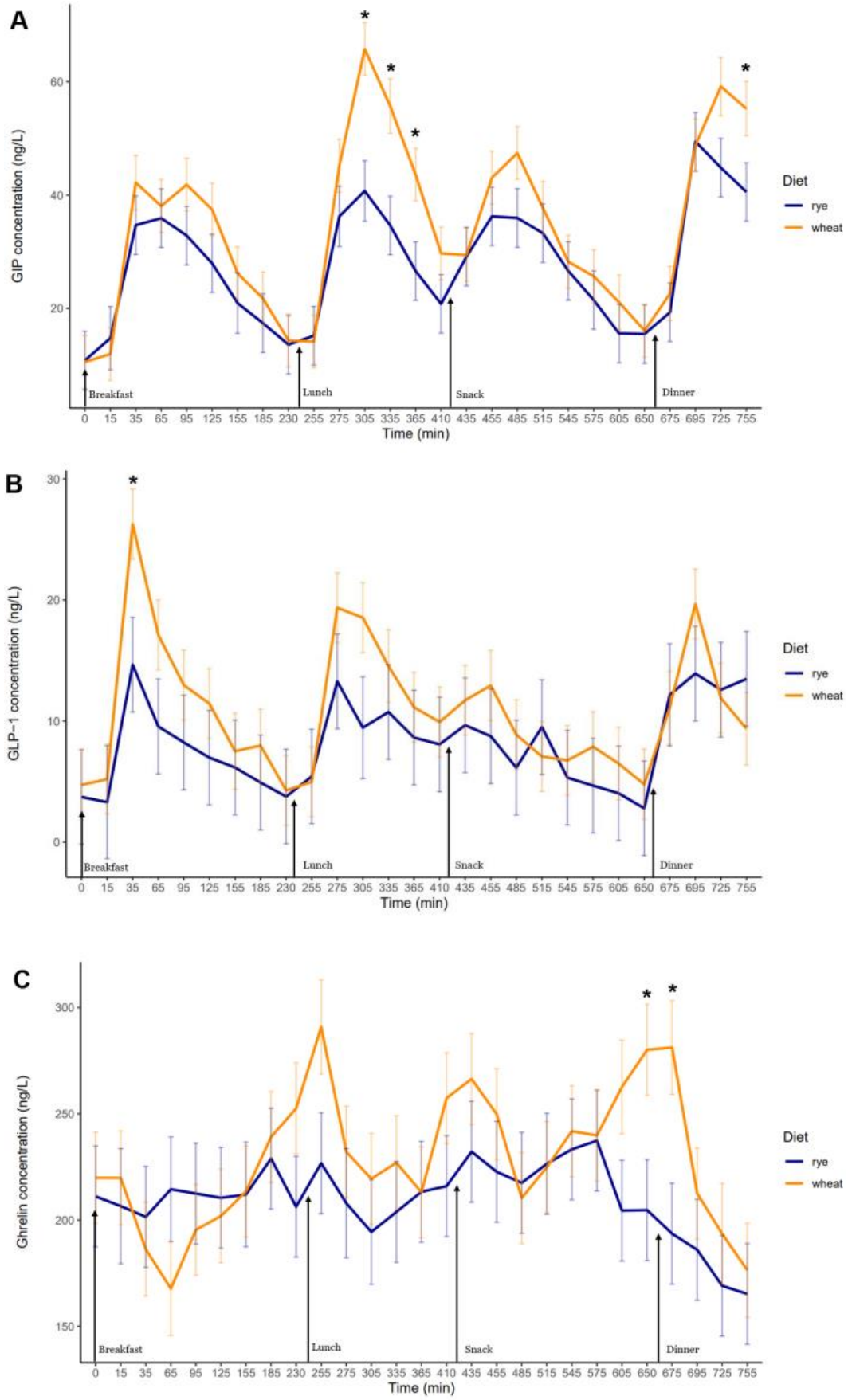

Figure 5.5.A. Gut hormone responses: GIP (A), GLP-1 (B) and ghrelin (C) measured as mean concentrations for rye- and wheat- based diets 0–755 min. Significant differences between diets at certain timepoints are indicated by *($p < 0.05$). Data are presented as estimated marginal means ± SEM, n = 20.

Participants in the rye-group had significantly higher baseline HOMA-IR compared to those following the wheat-based diet for this intervention day with continuous blood sampling. The average HOMA-IR was calculated to 4.7 in the rye-group compared with 1.9 in the wheat-group (Table 5.1.3). As this discrepancy in metabolic status may influence glucose metabolism as well as incretin secretion, we opted to do a sensitivity analysis where hormone data were normalized to the participants baseline HOMA-IR.

Table 5.5.A shows model estimates for the HOMA-IR adjusted analysis in parentheses. Incretin hormone responses changed drastically in this analysis, showing 61% (p = 0.015) lower whole-day GLP-1 response to the rye vs wheat-based diet and similar contrasts for the lunch and snack postprandial periods. Whole-day GIP was also 40% (p = 0.03) lower following the rye vs wheat-based diet, while ghrelin results were unchanged in the sensitivity analysis. Mean GLP-1 concentrations from the sensitivity analysis, shown in **Figure 5.5.B**, can be compared with the unadjusted mean concentrations in Figure 5.5.A.

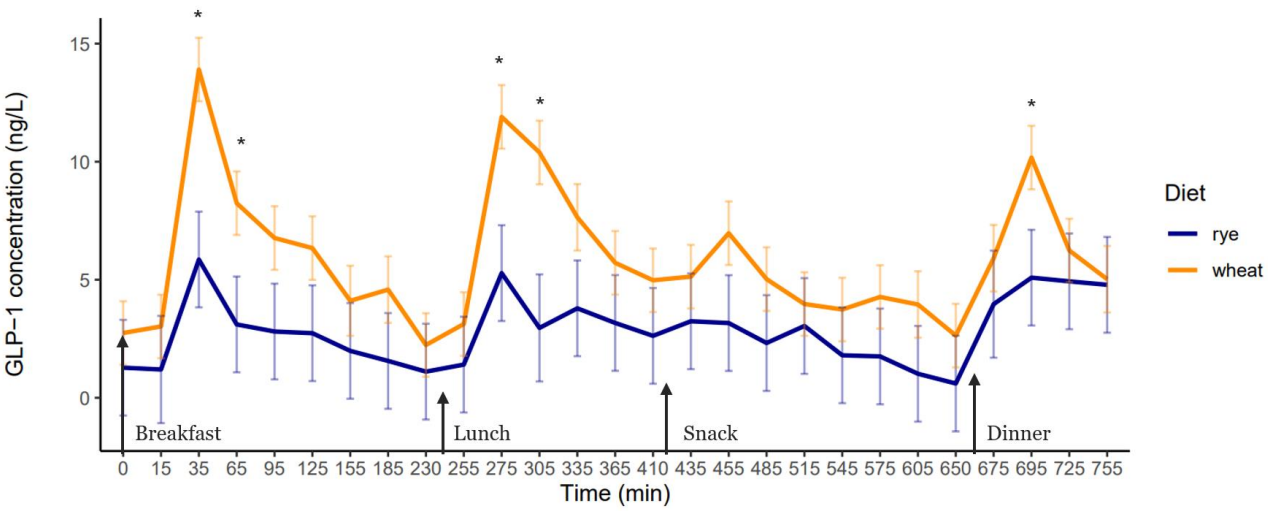

Figure 5.5.B. GLP-1, measured as mean concentrations for rye- and wheat- based diets 0–755 min. Model normalized to baseline HOMA-IR. Significant differences between diets at certain timepoints are indicated by *****($p < 0.05$). Data are presented as estimated marginal means ± SEM, n = 20.

In the VASA-home trial, subjective appetite scores indicated increased fullness and reduced postprandial hunger as well as lower ghrelin concentrations following the rye-based dinner. These results are in line with *Rósen et al.* reporting reduced postprandial ghrelin levels in two acute meal studies, evaluating effects of wholegrain rye vs refined wheat foods in normal weight participants [24,62]. However, neither *Hartvigsen et al.* [21] or *Heiononen et al.* [86], were able to demonstrate differences in postprandial ghrelin response to wholegrain rye vs refined wheat foods in participants with obesity. These conflicting results may again reflect impaired appetite signaling in individuals with overweight and obesity [25].

Few studies have evaluated the incretin response to whole grains versus refined grains, with even fewer focusing specifically on wholegrain rye. Two crossover trials, evaluating postprandial

responses to meals composed solely of rye products showed effects on incretins. *Hartvigsen et al*. demonstrated reductions in GLP-1 following breakfasts with wholegrain rye porridge vs semolina porridge. *Juntunen et al*. [30] showed lower GLP-1 and GIP concentrations after wholegrain rye kernel bread compared with refined wheat bread, but no difference measured as iAUC. There are some indications of postprandial effects of wholegrain rye on incretin response, but studies are limited and difficult to compare due to varying designs and study populations. Our study assessing rye foods as part of a complex diet show indications of reduced postprandial GLP-1 and GIP when differences in metabolic status between group were considered (Table 5.4.3.B). These results should be interpreted with caution but provide insights in incretin responses to cereals foods of wholegrain rye and refined wheat. In future study designs baseline metabolic status and insulin resistance should be considered.

In summary, substituting refined wheat with wholegrain rye foods resulted in no overall differences in postprandial GIP or GLP-1 responses, though the rye-based dinner reduced ghrelin levels, corresponding with decreased self-reported hunger. However, when accounting for baseline differences in HOMA-IR, the rye-based diet resulted in significantly lower GLP-1, and GIP responses compared to the wheat-based diet.

5.6. Linking subjective appetite with plasma insulin and glucose

In the RyeWeight2 study, an exploratory analysis of associations with fasting clinical markers and subjective appetite showed a discrepancy between the rye- and wheat-based diet. Correlations between fasting glucose, insulin and calculated HOMA-IR and measures of appetite are presented in **Table 5.6**. No correlations were observed in the rye-group. However, in the wheat-group, fasting insulin and HOMA-IR were positively correlated with fullness and inversely correlated with hunger and desire to eat. It should be noted that fasting glucose, insulin and HOMA-IR did not differ between the rye- and wheat-group at either baseline, week 6 or week 12. No correlations were observed for fasting glucose.

Table 5.6. Pearson correlation (r) between fasting glucose, insulin and HOMA-IR and subjective appetite measures: fullness, desire to eat and hunger measured as tAUC in the RyeWeight2 study.

	Fullness tAUC	**Hunger tAUC**	**Desire to eat tAUC**
		Rye-group	
Fasting glucose (mmol/L)	r = 0.05, p = 0.355	r = 0.04, p = 0.477	r = 0.06, p = 0.269
Fasting insulin (uUl/mL)	r = 0.08, p = 0.147	r = -0.005, p = 0.933	r = 0.02, p = 0.757
HOMA-IR	r = 0.08, p = 0.135	r = -0.003, p = 0.955	r = 0.02, p = 0.739
		Wheat-group	
Fasting glucose (mmol/L)	r = 0.04, p = 0.45	r = -0.06, p = 0.29	r = -0.08, p = 0.16
Fasting insulin (uUl/mL)	r = 0.11, p= 0.041*	r = -0.15, p = 0.0063**	r = -0.19, p = 0.00057***
HOMA-IR	r = 0.12, p = 0.0346*	r = -0.15, p = 0.005**	r = -0.19, p = 0.00054***

Data from baseline, week 6 and week 12 are included in the correlation analysis. HOMA-IR (Homeostatic Model Assessment for Insulin Resistance). Significant correlations are indicated by *p < 0.05, ** p < 0.01, ***p < 0.001.

Figure 5.6 illustrates the associations between subjective appetite and glucose response following rye- and wheat-based breakfasts in the VASA-home trial. Postprandial glucose $iAUC_{0\text{-}4h}$ and glucose $rise_{0\text{-}2h}$ were positively associated with fullness and inversely associated with hunger and desire to eat across diets. However, associations with glucose $rise_{0\text{-}2h}$ and appetite responses were driven by wheat-based breakfasts. Additionally, postprandial insulin tAUC was strongly associated with fullness and inversely associated with hunger and desire to eat following wheat-based breakfasts (Paper III). No such associations were observed for rye-based breakfasts. A similar trend was observed for wheat-based lunches; however, the snack meals and dinners did not demonstrate the same pronounced, diet-specific associations.

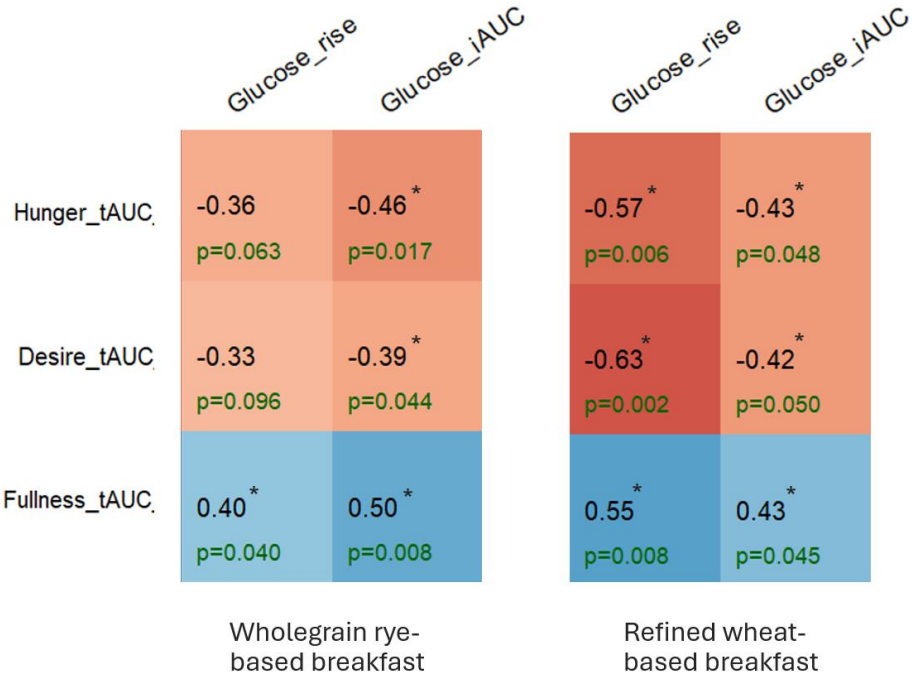

Figure 5.6. Associations for subjective appetite and glucose response following rye- and wheat-based breakfasts. Spearman rank correlation (r); glucose $iAUC_{0\text{-}4h}$, glucose $rise_{0\text{-}2h}$ and subjective appetite measures: fullness, desire to eat and hunger measured as $tAUC_{0\text{-}4h}$. Significant correlations are indicated by *(p < 0.05), *n = 21*.

The glucostatic theory, proposed by *Mayer et al*. [152] in the 1950s, suggests a link between blood glucose levels and appetite, with high glucose concentrations signaling satiety and low levels triggering hunger. However, results are conflicting and evidence that postprandial glucose is the primary driver of satiety and subsequent energy intake remains elusive [153,154]. In a cross-over trial by *Flint et al.*[154] glycemic responses were uncorrelated to appetite, whereas postprandial insulin was associated with greater fullness and less hunger in lean male participants. These findings were reinforced in a meta-analysis by *Flint et al.* [155], which emphasized the insulin-

appetite association in normal-weight individuals, as opposed to those who are overweight or obese. In the VASA-home trial, we observed similar associations between insulin and appetite, as well as between postprandial glucose and appetite, in individuals with overweight and obesity. Unlike *Flint et al.*, who found that these associations disappeared after adjusting for the energy content of test meals, the VASA-home trial used energy-matched test meals by design, which strengthens the observed associations. The reason we observe more pronounced associations following wheat-based meals is likely due to higher insulemic and glycemic responses compared with rye-based meals, as reported in Paper III.

In summary, fasting insulin and HOMA-IR were positively correlated with fullness and inversely correlated with hunger and desire to eat in the RyeWeight2 study. Similar associations were observed in the VASA-home trial for postprandial insulin and glucose and postprandial appetite measures. Interestingly, associations were more pronounced following wheat-based meals in the VASA-home trial and only shown within the wheat-group in the RyeWeight2 study.

5.7. Effect of wholegrain rye on body weight and body composition

In the RyeWeight2 study, participants lost 3.2kg of body weight following the rye-based diet for 12 weeks However, participants in the wheat-group lost 2.9kg of body weight and there was no significant difference between the two groups at either 6- or 12-week follow-ups. Additionally, the change in body fat mass measured by DXA at 6 and 12 weeks were similar between groups (**Figure 5.7**). There were no differences between groups in any of the anthropometric measures, including waist and hip circumference, sagittal height and additional body composition measures by DXA.

These results are in contrast with the RyeWeight1 study [37], where participants in the rye-group lost more weight and body fat mass compared to participants in the wheat-group. Interestingly, weight loss in the rye-groups were similar between the two studies, while the wheat-group in RyeWeight2 lost significantly more weight compared to the wheat-group in RyeWeight1. Study designs and intervention diets were identical between the two studies and the same criteria for participant inclusion and exclusion were employed. The RyeWeight2 study was mainly conducted to confirm results from the RyeWeight1 study in a different geographic population, to support a potential EFSA health claim (article 13.5) application on rye-fibre and body weight loss. In both RyeWeight studies, participants completed a 2-week run-in period before the 12-week parallel phase. In the RyWeight2 study, participants lost on average 1.7kg across intervention groups during this period.

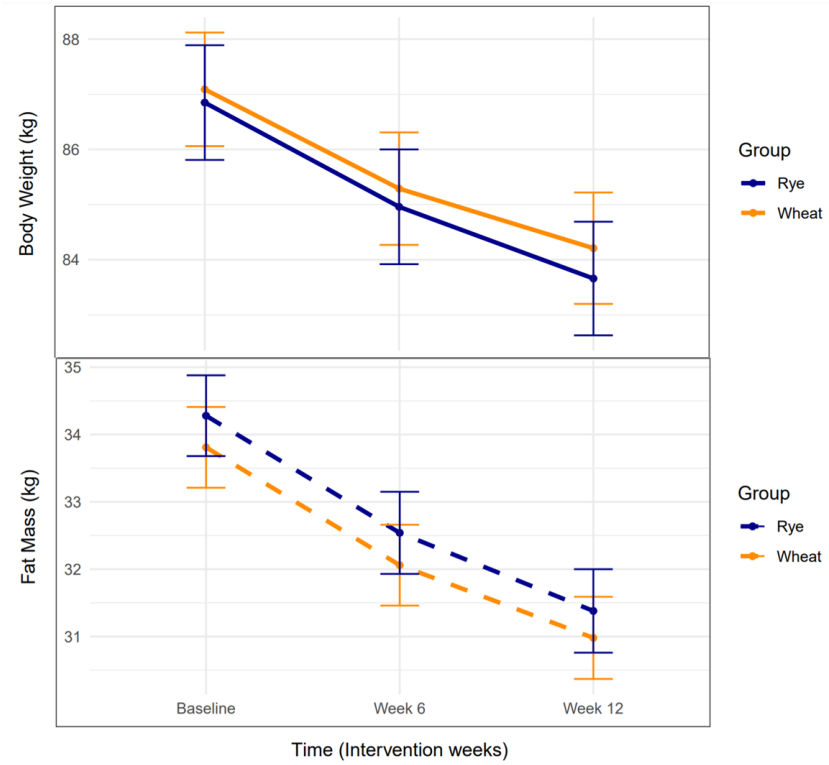

Figure 5.7. Body weight and fat mass change after 6 and 12 weeks for wholegrain rye and refined wheat-based interventions. Data are presented as estimated marginal means ± SEM, not adjusted for baseline. Data are presented as complete case analysis, n: rye=113, wheat=116.

Very few studies have been published on this topic, one of them being a 6-week intervention by *Suhr et al.* [104]. Participants with overweight or, obesity replaced habitual cereals with wholegrain rye, wholegrain wheat or refined wheat. The rye-group lost more body weight and fat mass compared to the refined wheat-group. In this trial, participants consumed ad libitum of the intervention foods, and it is reported that participants consumed more (~200kcal/day) of the intervention foods in the wheat-group. Intervention studies with wholegrain rye cereals have consistently shown increased satiety compared to refined wheat cereals [21–24,79]. Energy intake is primarily controlled by individual appetite sensations [156] and replacing habitual refined cereals with wholegrain rye cereals pose as a dietary strategy to control energy intake and body weight. There are a few studies showing associations with body weight change and appetite response [157–159]. However, to date there are no whole grain intervention studies establishing such associations with appetite and body weight changes.

In summary, participants lost body weight and fat mass after 12 weeks on hypocaloric diets on incorporating wholegrain rye or refined wheat foods. However, no differences were observed between intervention groups, contrasting with findings from the RyeWeight1 study, showing greater weight loss following 12 weeks of the wholegrain rye-based diet.

5.8. Gut microbiota and SCFAs – implications of wholegrain rye

In the RyeWeight2 study, gut microbiota composition was analyzed at baseline and after 12-weeks of intervention in fecal samples. Bacterial richness and Shannon's diversity index were reduced in the wholegrain rye versus refined wheat-based intervention, which contrasts with reported findings in studies assessing high fibre diets [160]. However, some studies have reported reduced bacterial richness, measured as α-diversity, in individuals with overweight and obesity following arabinoxylan-supplemented diets compared to controls [161]. Diet-induced changes in relative abundance of microbial species showed systematic differences between the rye and wheat-group (**Figure 5.8.A**). Species presented in Figure 5.8.A, are selected as high contributors to changes in relative abundance by a random forest algorithm. Selected species that showed relative high abundance after 12-weeks of the rye-based intervention include, *Bifidobacterium_adolescentis, Lacrimispora_amygdalina and Clostridium_sp_AF34_10BH,* while *Ruminococcus_torques, Romboutsia_timonensis and Ruthenibacterium_lactatiformans* showed relative low abundance compared to the wheat-based intervention group.

Relative increase in *Bifidobacterium adolescentis* and reductions in *Ruthenibacterium lactatiformans* have previously been reported in high-fibre and/or inulin supplemented interventions [162–164]. Supplementation with *Bifidobacterium adolescentis* have been shown to restore gut microbiota homeostasis and SCFA producing species, reducing inflammatory markers in mice with type 2 diabetes [165]. Furthermore, supplementation with *Lactobacillus acidophilus, Bifidobacterium bifidum*, and prebiotic fiber resulted in decreased fasting glucose levels and an increase in HDL cholesterol in patients with type 2 diabetes [166]. Both *Streptococcus salivarius* and *parasanguinis* are most abundant in the oral cavity and observed reductions in fecal samples in the rye-group may not have much health implications [167].

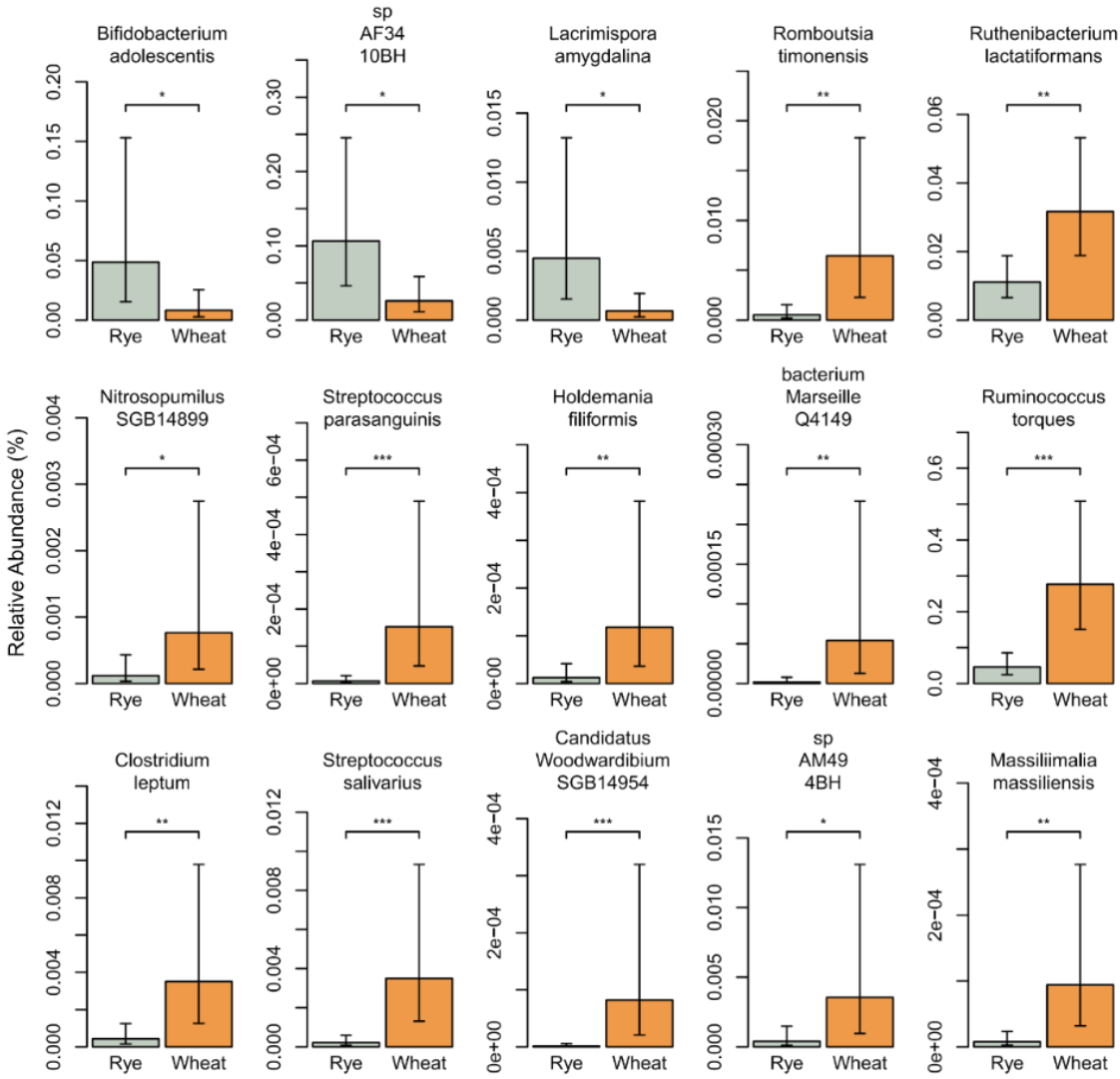

Figure 5.8.A. Bacterial species selected from Random Forest modelling, comparison of relative change for rye and wheat interventions. Data is presented with estimated marginal means and 95% confidence interval. Significant differences between diets are indicated by *p < 0.05, ** p < 0.01, ***p < 0.001.

Bacteria that differed between the rye- and wheat-group in the RyeWeight1 study [42] were selected for comparison. **Figure 5.8.B** shows relative differences in gut microbiota abundance at 12-weeks in the RyeWeight2 study for the selected bacteria. Reductions in *Anaerotruncus, Anaerofilum and Holdemania* at genus level and *Ruminococcus Torques* at species level were reported in both studies and are highlighted. Additionally, *Haemophilus* and *[Eubacterium] ventriosum group* showed non-significant trends in line with observations from the RyeWeight1 study. It should be noted that microbiota in the RyeWeight1 study was analyzed with 16S rRNA sequencing, while microbiota in the RyeWeight2 was analyzed by shot-gun metagenomic sequencing, allowing for species-level analysis to a greater extent.

Species that were decreased in rye- versus wheat-group *[Ruminococcus] torques group, Anaerotruncus, Anaerofilum,* and *Holdemania* have all been associated with inflammation, obesity, and/or elevated fasting glucose [168–172]. *Ruminococcus torques* has been associated with inverse function of gut barrier integrity, possibly through mucus degradation [173,174]. Gut

barrier function has in turn been linked to low-grade inflammation [175], which may be mediating effects on CRP reductions observed in both RyeWeight studies following the rye-based diet [37]. We have shown in two large interventions that effects of wholegrain rye foods compared with wheat foods on microbiota composition were similar (Figure 5.8.B). The changes in genus and species observed with the incorporation of wholegrain rye foods into the habitual diet indicate beneficial effects on cardiometabolic health.

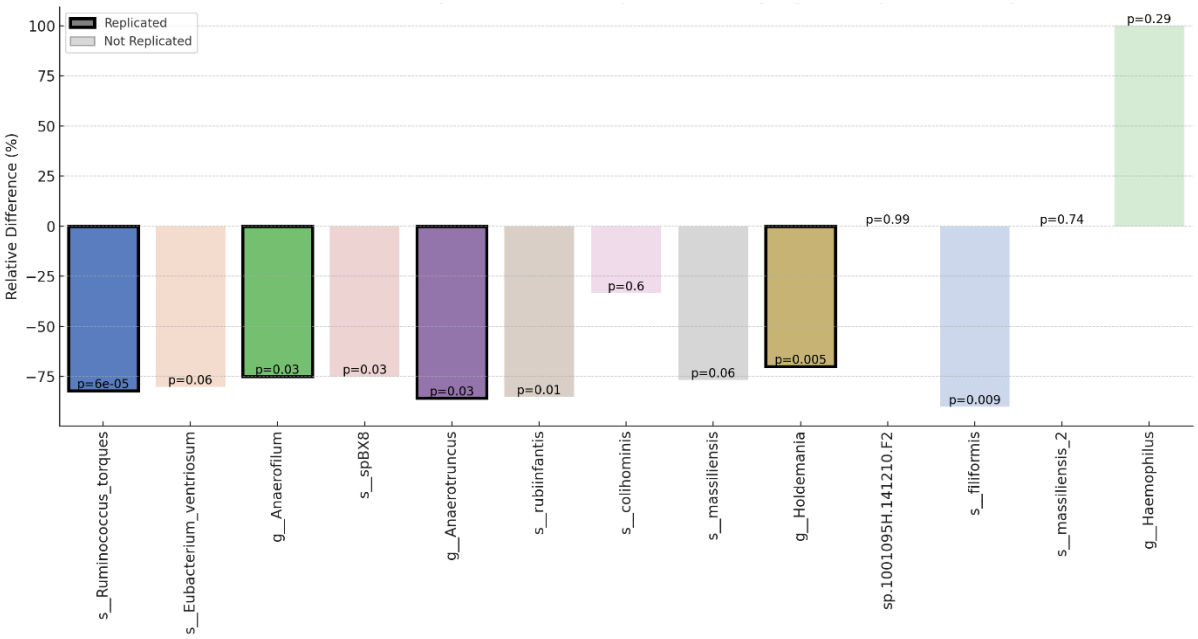

Figure 5.8.B. Difference in relative abundance after 12 weeks as %. Bacteria that differed significantly between rye and wheat in the RyeWeight1 study were included, and intervention effects from the RyeWeight2 study presented. Species that showed significant differences at 12 weeks in both studies are highlighted.

Plasma butyric acid was higher at 6 and 12 weeks in individuals with overweight and obesity following rye vs wheat-based diets (**Table 5.8**). Butyrate levels remained unchanged in the wheat-group throughout the intervention, resulting in significant increases in the rye-group relative to the wheat-group at both 6- and 12-week follow-up. The absolute increase in plasma concentration was 32% ($p = 0.003$) and 38% ($p < 0.001$) after 6 and 12 weeks of rye-based intervention respectively and were similar in the RyeWeight1 study [42].

Across our previous RyeWeigth1 study and now the RyeWeright2, involving a total of 436 individuals, we consistently demonstrated significant increases in plasma butyrate levels when consuming wholegrain rye compared to refined wheat foods as part of a complex diet. Furthermore, acetate levels decreased in the wheat-group, leading to relatively higher acetate levels in the rye-group at week 12 (Table 5.3). Similar changes were observed in the RyWeight1 study, resulting in significant differences at week 6.

Intervention studies investigating effects of whole grains on SCFA levels show inconclusive results [116,118,176]. Diet induced changes of SCFA has been reported to be highly influenced by fiber type, structure and dose [177]. In healthy volunteers (BMI ~ 21), *Damen et al*. showed that arabinoxylan fortified bread vs white wheat bread increased fecal butyrate and trends towards increased acetate were observed [122]. *Vetrani et al*. reported increased propionate with mixed whole grains vs refined cereals in participants with metabolic syndrome [121]. Recently, plasma SCFA were shown to be inversely correlated with BMI, while fecal SCFA did not show such associations [123]. To our knowledge the RyeWeight studies are the first intervention studies to show consistent and relative increases in plasma butyrate following whole grain vs refined grain interventions in overweight and obese individuals.

In summary, across both our RyeWeight1 and RyeWeight2 studies, involving a total of 436 individuals, we observed distinct shifts in gut microbiota composition and plasma SCFAs. The rye-group showed reductions in specific genera and species associated with negative health outcomes, along with increased plasma acetate and butyrate, which may have implications for cardiometabolic health.

Table 5.8. Plasma short chain fatty acids by diet

		Week 0*	Week 6*	Week 12*	Δbetween groups week 6**	Δbetween groups week 12**
Formic acid	Rye	66.9 (60.1;73.6)	69.9 (63.1;76.6)	69.7 (62.9;76.4)	0.4266	0.9255
	Wheat	66.2 (59.7;72.7)	67.6 (61.0;74.1)	70.1 (63.6;76.6)		
Acetic acid	Rye	60.6 (53.2;68.0)	65.7 (58.3;73.2)	62.2 (54.8;69.5)	0.2126	**0.0349**
	Wheat	66.5 (59.3;73.7)	64.0 (56.9;71.2)	57.1 (49.9;64.3)		
Propionic acid	Rye	0.60 (0.52;0.68)	0.61 (0.53;0.69)	0.62 (0.54;0.70)	0.6902	0.6035
	Wheat	0.53 (0.46;0.61)	0.58 (0.51;0.66)	0.59 (0.52;0.67)		
Butyric acid	Rye	0.37 (0.30;0.44)	0.49 (0.42;0.56)	0.51 (0.44;0.57)	**0.0048**	**0.0027**
	Wheat	0.41 (0.34;0.47)	0.40 (0.33;0.47)	0.42 (0.35;0.48)		
Isobutyric acid	Rye	0.15 (0.14;0.17)	0.16 (0.14;0.18)	0.15 (0.14;0.17)	0.5333	0.6082
	Wheat	0.15 (0.13;0.16)	0.15 (0.13;0.16)	0.15 (0.14;0.17)		
Succinic acid	Rye	2.22 (2.08;2.35)	2.22 (2.08;2.35)	2.27 (2.13;2.40)	0.2131	0.9875
	Wheat	2.30 (2.17;2.43)	2.36 (2.23;2.49)	2.30 (2.17;2.43)		
Valeric acid	Rye	0.05 (0.04;0.06)	0.04 (0.03;0.05)	0.05 (0.04;0.06)	0.5914	0.8679
	Wheat	0.04 (0.03;0.05)	0.05 (0.04;0.05)	0.05 (0.04;0.06)		
Isovaleric acid	Rye	0.51 (0.46;0.57)	0.53 (0.47;0.58)	0.57 (0.51;0.62)	0.8146	0.3538
	Wheat	0.48 (0.42;0.53)	0.51 (0.45;0.56)	0.52 (0.47;0.58)		
Caprionic acid	Rye	0.14 (0.11;0.17)	0.16 (0.13;0.19)	0.18 (0.15;0.20)	0.3683	0.7404
	Wheat	0.17 (0.15;0.20)	0.18 (0.16;0.21)	0.18 (0.15;0.20)		

* Geometric mean and 95% confidence intervals, not baseline adjusted. ** Difference between the groups at week 6/week 12 in a baseline adjusted model. Data are presented as complete case analysis. Significance level is $p < 0.05$, significant p-values are marked in bold font. n: rye=113, wheat=116.

5.9. Inflammatory markers and wholegrain rye

In the VASA-home trial, GlycA and SPC levels were slightly elevated in participants consuming meals with wholegrain rye foods compared with refined wheat foods (**Table 5.9.A**). Also, GlycB showed a similar trend, while postprandial CRP concentrations were similar between the rye- and wheat-group.

Table 5.9.A. Continuously measured inflammatory markers in plasma

	Z-score difference between diets	P-value difference between diets
GlycA	0.36 ± 0.13	0.014*
GlycB	0.35 ± 0.17	0.054
SPC	0.27 ± 0.13	0.044*

Inflammatory markers GlycA, GlycB and SPC measured as Z-score difference of diet-induced inflammation measured over the whole day 0-725min (the wheat diet is reference = 0). Data is presented with estimated marginal means ± SEM. Significant differences between diets are indicated by *p < 0.05. GlycA, glycoprotein N-acetylation A; GlycB, glycoprotein N-acetylation B; SPC, supramolecular phospholipid composite peak.

In the RyeWeight2 study fasting CRP decreased in the rye-group, while remaining unchanged in the wheat-group resulting in a 17% difference at 12 weeks. This effect was present also at 6 weeks when outliers (CRP >10.00 mg/L) were excluded from analysis. We observed slight reductions of inflammatory markers GlycA, GlycB, and SPC, with similar reductions in both the rye- and wheat-group after 12 weeks (**Table 5.9.B)**.

Table 5.9.B. Continuously measured inflammatory markers in plasma

	Z-score difference between diets	P-value difference between diets
GlycA	0.16 ± 0.09	0.09
GlycB	0.16 ± 0.09	0.09
SPC	0.13 ± 0.08	0.12

Inflammatory markers GlycA, GlycB and SPC measured as 12-week Z-score difference between diets (the wheat diet is reference = 0). Data is presented with estimated marginal means ± SEM derived from model adjusted for baseline and bodyweight change. Significant differences between diets are indicated by *p < 0.05. GlycA, glycoprotein N-acetylation A; GlycB, glycoprotein N-acetylation B; SPC, supramolecular phospholipid composite peak.

Observational data have shown an association between high whole grain intake and lower CRP levels [87], and results from intervention studies comparing whole grain versus refined grain foods indicate reduction in CRP and IL-6 [89,92]. Recently, we showed reduced fasting CRP levels after 12-weeks of wholegrain rye foods compared with refined wheat foods in the RyeWeight1 study [37]. In that study, differences in body weight between the rye- and wheat-group may partly have explained effects on CRP. In the VASA-home trial no differences in postprandial CRP

concentrations were observed. Short-term effects of whole grains on postprandial CRP have rarely been measured and in the current trial these measures were exploratory. With 21 individuals in a parallel design, we might not be power to evaluate postprandial effect on CRP.

Some studies have shown beneficial effects of wholegrain rye foods on low grade inflammation. *Xue et al*. found that CRP was lower in participants consuming fermented wholegrain rye compared with refined wheat bread after 12 weeks [93]. Interestingly, this effect was observed only in participants with overweight, suggesting that the impact of wholegrain rye interventions on CRP may be more pronounced in individuals with overweight compared to those who are lean. *Kallio et al*. showed that inflammatory markers in adipose tissue and plasma concentrations of IL-1β and IL-6 were reduced after 12 weeks of whole meal rye compared with whole meal oat and wheat breads, while plasma CRP remained unchanged [94]. The results in the RyeWight2 are aligning with findings from *Xue et al*. [93] and confirming observed effects on CRP in the RyeWeight1 study in similar populations with overweight and obesity [37]. Furthermore, inflammatory markers GlycA, GlycB and SPC were measured at baseline and 12-week following in the RyeWeight2 study and measured continuously in the postprandial phase in the VASA-home trial. We found effects with increased GlycA and SPC in the VASA-home trial after meals with wholegrain rye foods, while small reductions in the RyeWeight2 study were similar between diets and reflecting weight loss after 12 weeks of intervention.

Wyatt et al. recently suggested that postprandial GlycA may provide a more accurate indication of an individual's systemic metabolic status compared to fasting measures of GlycA and IL-6 [99]. However, studies evaluating postprandial GlycA in this context and connecting postprandial GlycA levels to traditional clinical markers are lacking. The VASA-home trial was not primarily designed to evaluate GlycA and observed increase in GlycA following consecutive meals with wholegrain rye foods should be interpreted with caution. Based on our observations in the RyeWeight 2 study, postprandially elevated GlycA does not translate to long-term effects.

In summary, reductions in CRP after 12-weeks of the wholegrain rye versus refined wheat-based diet shown in the RyeWeight2 study confirms findings from the RyeWeight1 study and suggests beneficial effects on subclinical inflammation. No intervention effects on fasting GlycA, GlycB and SPC were observed in the RyeWeight2 study, however postprandial levels were increased following consecutive rye-based melas in the VASA-home trial.

5.10. Determinants of weight loss and metabolic risk markers

As reported in Paper IV, weight loss did not differ between the rye and wheat-group at 12 weeks in RyeWeight2 study. However, there was substantial inter-individual variability in body weight change across diet (**Figure 5.10.A**). Similar variability in weight loss was also observed in the RyeWeight1 study [37] and has been reported in other hypocaloric dietary interventions [36]. Hence, factors predicting successful body weight and fat mass loss were examined

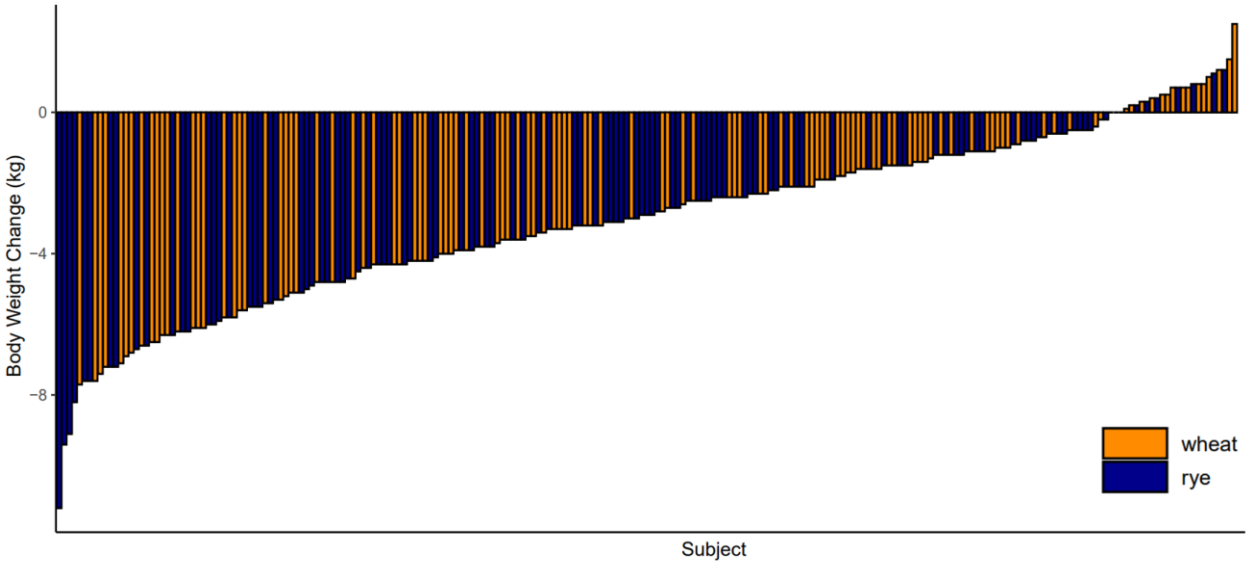

Figure 5.10.A. 12-week body weight change in the RyWeight2 study illustrated by participants.

Overall, we did not observe any differences between the wholegrain rye and refined wheat-group when assessing subjective appetite and no associations with weight loss were found in the RyeWeight2 or RyeWeight1 study. These findings contradict our initial hypothesis that greater weight loss with rye cereal would be driven by its appetite-suppressing properties.

In contrast to the RyeWeight1 study, no microbial genus, species or strains showed associations with weight or fat mass loss in either the rye or wheat-group. This outcome was consistent across both univariate analysis and machine learning modeling. Although, plasma SCFA at baseline showed associations with body weight change, as acetate was inversely associated with weight and fat mass across diets at 12 weeks. However, this association was more pronounced in the rye-group (**Figure 5.10.B**). Participants with high vs low (50:50) baseline acetate in the rye-group lost 4kg vs 2.4kg of body weight, while in the wheat-group, the weight loss was 3.2 kg and 2.6 kg, respectively (**Table 5.10.A**). Acetate is the most abundant SCFA in the human colon and in plasma [178,179] and has been shown to increasing energy expenditure and fat oxidation through secretion of enteroendocrine gut hormones [180]. Additionally, baseline isovaleric acid also showed an inverse association with weight and with fat mass at 12-weeks, in the wheat-group specifically. This exploratory analysis in the RyeWeight2 study is the first to show that baseline plasma acetate predicts weight loss in individuals with overweight and obesity, suggesting

individuals with high plasma acetate concentrations benefit more from wholegrain rye cereals as part of a hypocaloric diet for weight loss.

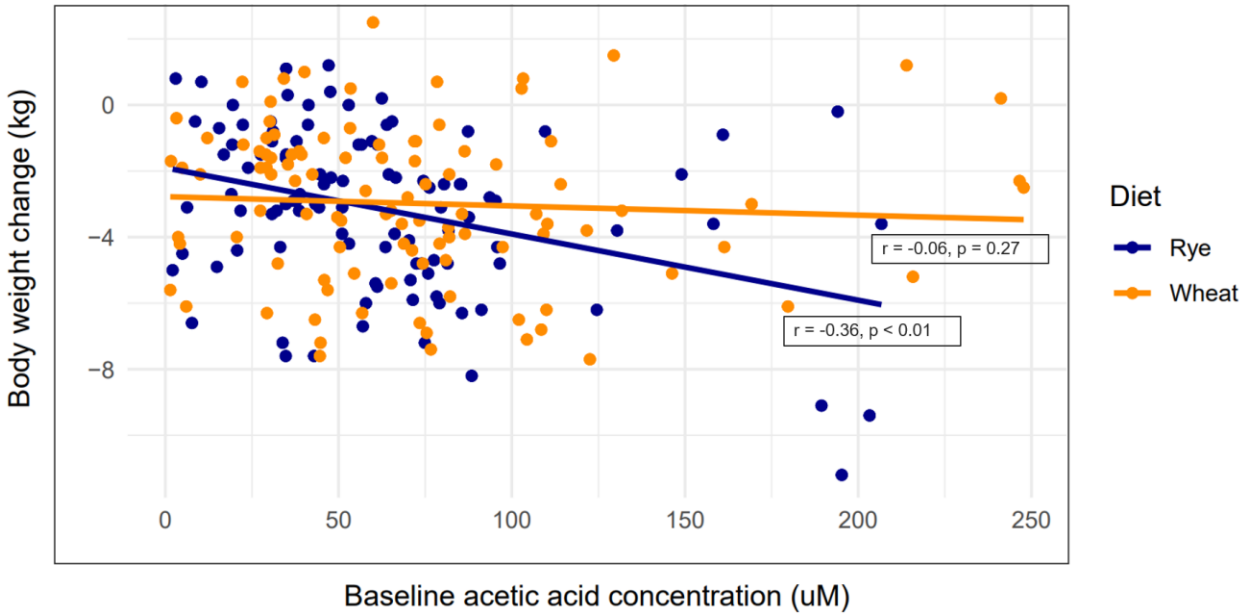

Figure 5.10.B. Associations with baseline plasma acetic acid concentration and body weight change by diet in the RyeWeright2 study. Spearman's correlation coefficient (r) and slopes (blue = wholegrain rye, orange = refined wheat) derived from linear regression.

Table 5.10.A. Baseline plasma acetic acid and 12-week change of body weight and body fat mass by diet.

	Acetic acid - Low	Acetic acid - High	p-value
		Rye-group (n = 113)	
Body weight (kg)	-2.42 ± 0.18	-4.02 ± 0.20	**< 0.001**
Fat mass (kg)	-2.29 ± 0.15	-3.59 ± 0.16	**< 0.001**
		Wheat-group (n = 116)	
Body weight (kg)	-2.57 ± 0.19	-3.23 ± 0.17	**0.01**
Fat mass (kg)	-2.65 ± 0.16	-3.03 ± 0.15	0.09

Data are presented as baseline adjusted estimated marginal means ± SEM. Significant p-values are highlighted in bold font (p-value < 0.05).

Furthermore, fasting insulin and baseline HOMA-IR were associated with weight and fat mass loss within the wheat-group. Participants in the wheat-group with baseline HOMA-IR < 2.0 lost on average 1kg more of body weight as compared to those with HOMA-IR > 2.0 (**Table 5.10.B**). When comparing the rye groups across the two RyeWeight studies, HOMA-IR levels were similar. However, comparing wheat groups, HOMA-IR was 19% lower in RyeWeight2 compared to the RyeWeight study (**Table 5.10.C**). This marked difference in HOMA-IR may partly explain why participants in the RyeWeight2 wheat-group experienced significantly greater weight loss than those in RyeWeight1. Baseline inflammation status emerged as a diet-specific determinant of body composition change, with both baseline CRP and GlycA levels showing associations with fat mass loss (Paper IV).

In summary, our data across two large studies suggests that gut microbiota is not the primary determinant of diet-induced weight loss, but other metabolic factors, such as subclinical inflammation and progression towards insulin resistance playing a more significant role. Inflammatory status as well as HOMA-IR at baseline seem to influence participants ability to lose weight in the wheat-group only, while weight loss was similar independent of these markers in the rye-group. This suggests that individuals with overweight or obesity, who exhibit elevated inflammation and advancing insulin resistance, may achieve greater weight loss by substituting refined cereals with wholegrain rye.

Table 5.10.B. Baseline HOMA-IR and 12-week change of body weight and body fat mass by diet.

	Rye-group (n = 113)	**Wheat-group (n = 116)**	**p-value**
		HOMA-IR < 2.0	
Body weight (kg)	-2.71 ± 0.18	-2.70 ± 0.19	0.95
Fat mass (kg)	-2.82 ± 0.16	-3.11 ± 0.17	0.18
		HOMA-IR > 2.0	
Body weight (kg)	-3.67 ± 0.18	-2.65 ± 0.20	**0.0002**
Fat mass (kg)	-3.10 ± 0.15	-2.57 ± 0.16	**0.01**

Data are presented as baseline adjusted estimated marginal means ± SEM. Significant p-values are highlighted in bold font (p-value < 0.05).

Table 5.10.C. Baseline characteristics in the RyeWeight1 and RyeWeight1 study.

	RyeWeight1		**RyeWeight2**	
	Rye-group	Wheat-group	Rye-group	Wheat-group
Number of participants	108	99	113	116
Number of women (%)	56	63	64	70
Age (years)	56.8 ± 9.4	57.3 ± 9.6	56.1 ± 9.6	54.0 ± 10.3
Weight (kg)	88.8 ± 12.8	89.1 ± 12.3	86.9 ± 10.4	87.1 ± 11.7
BMI (kg/m2)	29.8 ± 2.5	30.3 ± 2.5	29.7 ± 2.1	29.5 ± 2.4
Fat mass (kg)	34.0 ± 6.5	35.8 ± 7.2	34.3 ± 5.6	33.8 ± 7.1
Lean mass (kg)	50.9 ± 10.8	49.5 ± 10.1	49.7 ± 9.4	50.3 ± 9.6
Systolic BP (mmHg)	126.3 ± 14.6	123.8 ± 15.6	124.0 ± 11.2	125.2 ± 12.3
Glucose (mmol/L)	5.5 ± 0.5	5.6 ± 0.6	5.3 ± 0.6	5.2 ± 1.5
Insulin (mIU/l)	10.0 ± 5.3	10.9 ± 5.2	10.4 ± 7.4	9.6 ± 5.6
HOMA-IR	2.5 ± 1.5	2.8 ± 1.6	2.6 ± 2.1	2.3 ± 1.5
Triglyceride (mmol/l)	1.1 ± 0.4	1.2 ± 0.5	1.0 ± 0.3	1.0 ± 0.4
Total cholesterol (mmol/l)	4.7 ± 0.9	4.8 ± 1.0	4.9 ± 0.8	4.8 ± 1.0
LDL cholesterol (mmol/l)	3.1 ± 0.8	3.1 ± 0.8	3.0 ± 0.7	3.0 ± 0.9
CRP (mg/l)*	2.0 ± 2.0	1.9 ± 1.5	1.8 ± 1.6	1.6 ± 1.3

Data are presented as means ± SD. Abbreviations: BP, blood pressure; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low density lipoprotein; CRP, C-reactive protein. * Values >10.00 mg/L has been removed.

6 GENERAL DISCUSSION

Whole grain particle size and glycemia

In the Whole-grain Milling & Glycemia trial, consumption of largely intact whole grains, compared to finely milled whole grains, resulted in reduced glucose iAUC and glycemic variability. Few studies have examined the impact of whole grain processing, specifically particle size, on glycemic response [35,145–147]. A comparable intervention by *Järvi et al*. [35] found effects on postprandial glucose iAUC using nutrient-matched diets differing in glycemic index. In contrast, our study demonstrated effects of whole grain particle size on glycemia in free-living participants consuming intervention foods as part of their habitual diet, thus enhancing the generalizability of the findings. Additionally, the use of CGM for continuous glucose monitoring allowed for the measurement of day-long glycemic variability, and this may be the first trial to show improved glycemic variability with intact versus finely milled whole grains. Our findings have relevance for nutritional guidelines for diabetes management, which currently recommends whole grains, but do not specify grain structure or particle size [181]. However, the European Association for the Study of Diabetes (EASD) has addressed this in its updated recommendations, stating that "the focus should be on minimally processed and largely intact whole grains, rather than products with finely milled whole grains" [182]. Further, these findings may also contribute to discussions on front-of-pack labeling to help consumers make informed decisions for improved glycemic control.

Self-reported appetite and metabolic effects of wholegrain rye

Traditionally, subjective appetite has been assessed with laboratory test meals, a highly resource-intensive procedure, where participants are required to travel and stay at the research facility. In addition, the generalizability of self-reported appetite in controlled clinical settings has been increasingly questioned, as these conditions differ significantly from normal free-living conditions. In the VASA-home trial we show good agreement for appetite responses between controlled clinical conditions and free-living conditions. This is highly relevant, since many studies assessing the health effects of foods and diets rely on self-sampling and use non-validated VAS through mobile apps to measure appetite responses to habitual diets [183]. Our evaluation of VAS in free-living conditions have contributed to validation of the method and could have implications for large intervention and cohorts with extensive sampling to assess appetite response to foods and meals.

Subjective appetite assessments were conducted in both the RyeWeight2 study and the VASA-home trial. In contrast to previous studies that have shown satiating effects of meals composed solely of wholegrain rye foods [21–24], our studies replaced commercially available refined grains with wholegrain rye, comprising approximately 1/3 of participants caloric intake. While this improves generalizability, it likely explains why no differences between diets were found.

Some effects on postprandial appetite were observed later in the day with higher satiety and reduced hunger following rye-based meals. The most pronounced effects were found after the rye-based dinner, likely due to accumulated fiber intake and increased SCFA production, which are key signaling molecules in appetite regulation [110–112]. Additionally, lower ghrelin levels

were measured after the rye-based dinner, indicating possible appetite-regulating properties of wholegrain rye. However, the observed effects on appetite are unlikely to impact prospective energy intake, as these observations did not reach the 15mm VAS threshold predictive of subsequent intake [78].

The VASA-home trial may be the first study to evaluate the effects of wholegrain rye foods on incretin hormones GIP and GLP-1 after several consecutive meals. Considering the pronounced effects on postprandial glucose, we expected effects on incretin hormones. However, glucose measures were conducted in cross-over design, while gut hormones were measured in venous blood samples collected in parallel design. In a sensitivity analyses, adjusting for group-differences in insulin resistance, significantly lower GIP and GLP-1 levels were found following most rye-based meals. Suggesting, effects of the rye foods on postprandial incretin response that were not captured in the unadjusted analysis in our relatively small intervention groups. In the current trial both rye- and wheat foods were made of finely milled flour. More intact whole grains could possibly influence incretin responses based on our findings in Paper 1. Furthermore, effects of wholegrain rye within complex meals and diets on incretin hormones should be investigated in a cross-over design or large intervention groups, enabling analysis stratified for markers of insulin resistance.

Effects of wholegrain rye on body weight and determinants of weight loss

The recent findings from the RyeWeight1 study with greater weight and fat mass loss with wholegrain rye versus refined wheat foods as part of a hypocaloric diet could not be confirmed in the present RyeWeight2 study. Despite relatively large intervention groups, some differences in participant baseline characteristics between rye and wheat groups were observed, including demographics, anthropometrics and clinical markers. In the RyeWeight2 study, the wheat vs rye-group was younger, with lower fat mass, BMI, insulin, and HOMA-IR, whereas the opposite was observed in the RyeWeight1 study, where the rye-group had lower fat mass, BMI, and HOMA-IR at baseline. These differences may partly explain the substantial weight loss observed in the RyeWeight2 wheat-group, where participants with baseline HOMA-IR < 2.0 lost on average, 1 kg more than those with HOMA-IR > 2.0. A possible explanation is that progressing insulin resistance leads to increased postprandial insulin secretion, inhibiting lipolysis for several hours, even at moderate insulin levels [184,185]. This effect is more pronounced in the wheat-group, as these foods release glucose and maltose more rapidly than the rye-based foods used in this study [61].

Interestingly, baseline plasma acetate showed inverse associations with body weight and fat mass at 12 weeks, primarily attributed to strong associations observed in the rye-group. Acetate appears to be the most promising SCFA, influencing appetite regulation, resting energy expenditure, and adiposity [180]. However, human studies are limited, with administration trough colonic infusion and supplementation trials showing mixed results on weight loss [180]. Our exploratory analysis of data from the RyeWeight2 study may be the first to indicate that plasma acetate could serve as a potential determinant of weight loss in individuals with overweight and obesity. We investigated whether species known to produce acetate were associated with weight or fat mass loss, but no such associations were found. Similarly, neither enterotypes nor bacterial genera at baseline predicted weight loss. While a few small studies have suggested that genus *Prevotella* may predict weight loss following fiber-rich rye diets, these findings have been post-hoc analyses from the same research group [38,39,105]. Both RyeWeight studies, with a

combined total of 436 individuals and gut microbiota analyzed via 16S rRNA and metagenomic shotgun sequencing, consistently show that gut microbiota was not a primary mechanism behind weight loss. Instead, other markers of metabolic status appear to be more important.

Gut microbiota, SCFAs and inflammatory markers

Consistently across the two RyeWeight studies, 12 weeks of wholegrain rye compared with refined wheat foods altered the gut microbiota with decreased relative abundance of [*Ruminococcus] torques, Anaerotruncus, Anaerofilum,* and *Holdemania*. These bacterial taxa have been linked to negative health outcomes such as inflammation, obesity, and elevated glucose levels [186–188]. In RyeWeight2 study, the rye-group specifically showed an increase in Bifidobacterium adolescentis, a species rarely reported in human studies, but effects on blood lipids [189,190], glucose metabolism and inflammatory markers [191] have been shown in animal studies.

No postprandial effects were observed on CRP, while inflammatory markers GlycA and SPC were slightly increased following rye-based meals in the VASA-home trial. Almost half of the participants in the VASA-home rye-group had GlycA levels that increased with 10% or more from fasting levels, while less than 10% of participants in the wheat-group experienced corresponding levels. These results contrast with the observed reductions in fasting inflammatory markers following wholegrain rye interventions [37,192]. However, GlycA has not been studied in the postprandial phase following wholegrain consumption before and it is thus difficult to draw any conclusions. Interestingly, no difference in fasting GlycA or SPC were observed in the RyeWeight2 study. Absolute levels were slightly reduced and associated with body weight loss in both groups. However, CRP levels were lower after the 12-week of rye- versus wheat-based diets, despite similar reductions in body weight. These findings confirm the results of the RyeWeight1 study and further support the evidence that wholegrain rye cereals reduce low-grade inflammation in individuals with overweight and obesity. Additionally, participants in the wheat-group with elevated GlycA and CRP levels experienced less weight and fat mass loss. In contrast, these associations were absent in the rye-group, where weight loss was consistent regardless of inflammatory status. This suggests that individuals with elevated low-grade inflammation may benefit more from wholegrain rye foods.

Overall, our studies suggest that substituting commonly consumed refined wheat cereals for wholegrain rye cereals can significantly reduce low-grade inflammation, improve glycemic control, and induce beneficial alterations in gut microbiota and SCFAs, potentially improving cardiometabolic health. Although no significant weight loss via appetite-regulating properties was observed, rye foods appear to support greater weight loss in individuals with elevated systemic inflammation and advancing insulin resistance.

7 LIMITATIONS

The studies conducted in this thesis all had strengths and limitations. Here, the limitations are outlined for the separate studies.

The Whole-grain Milling & Glycemia trial had several limitations that should be acknowledged. While the study demonstrated clinically relevant effects on glucose control in individuals with type 2 diabetes with high compliance, it is uncertain whether such adherence would be achievable in the general population. Participants in dietary interventions tend to be more health-conscious, likely resulting in better adherence, so the results should be interpreted with this in mind. Although the free-living setting strengthens the generalizability of the findings, it also meant that participants' meal timings were not strictly controlled. Meal timings used to calculate postprandial glucose iAUC was instead inferred from food diaries and habitual patterns through a hierarchical decision-making process. The study was not large enough to separately assess the effects of whole grain particle size across different grain types. Additionally, variations in diabetes progression and medication among participants limited our ability to assess effects at different levels of glycemic control or medication type. Finally, the 2-week intervention period was too short to evaluate effects on HbA1c, a critical clinical marker more closely linked to diabetes progression and potential remission.

The VASA home trial was designed to assess whether subjective appetite measurements using VAS in free-living conditions are equivalent to those obtained in a controlled clinical settings and sample size estimations calculated accordingly. The 5-way crossover design allowed for the evaluation of subjective appetite and glucose control following wholegrain rye and refined wheat-based diets. Gut hormones and inflammatory markers were measured from plasma samples collected during one intervention day, with participants randomized 50:50 to either the rye or wheat-based diets. With four consecutive meals and 27 samples taken throughout the day, we were confident that the number of participants was sufficient to assess gut hormone responses. However, the ability to detect effects on inflammatory markers remains uncertain, as no reference studies have evaluated postprandial variation in these markers. As such, the power to detect effects on incretin hormones, ghrelin, and inflammatory markers may have been limited, and these results should be interpreted with caution.

Additionally, the study was relatively small, and sex distribution skewed, hence effects of the intervention diet in men and women separately could not be considered. Furthermore, data on menstruation and hormonal contraceptive use were not collected, which may have influenced appetite sensations, gut hormones, insulin, and glycemic responses in female participants [193].

In **the RyeWeight2 study**, the wheat-group had a slightly higher protein intake, with the total energy percentage from protein being higher at both 6 and 12 weeks, as calculated from food diaries. This may have influenced appetite, as protein is known to have appetite-suppressing effects compared to other macronutrients [194]. The rye-based diet with high levels of dietary

fiber impacted gastrointestinal symptoms, which should be considered when implementing diets with similar amounts of wholegrain rye cereals.

Participants lost an average of 1.7 kg across intervention groups during the 2-week run-in period, which involved consuming refined wheat foods. Baseline fecal samples for gut microbiota composition were collected after this period, potentially introducing noise in our exploratory analysis of specific genera, species, or enterotypes as determinants of weight change. The same applies to plasma SCFA measurements taken at baseline. Despite the relatively large intervention groups, baseline characteristics differed slightly between the rye- and wheat-groups. Participants in the wheat-group were slightly younger, had a lower BMI, and higher lean mass, despite a greater proportion of women. Additionally, baseline insulin and HOMA-IR were significantly lower in the wheat-group, which is relevant given our observation that lower baseline HOMA-IR was associated with greater body weight and fat mass loss.

The study was conducted during the COVID-19 pandemic, but fortunately, it proceeded as planned without major deviations from the protocol. However, it's difficult to assess how the pandemic may have influenced participants, given the societal restrictions on movement and social gatherings. It is possible that the impact varied across age groups, though we lack sufficient data to detect significant differences between the intervention groups. Notably, physical activity related to commuting was significantly lower in RyeWeight2 compared to RyeWeight1 (conducted pre-pandemic), likely due to pandemic-related restrictions.

8 CONCLUSIONS

- Consuming largely intact whole grains resulted in lower postprandial glucose iAUC as well as improved measures of glycemic variability compared to wholegrain foods that were finely milled. Structural integrity of whole grains has effects on glucose control in individuals with type 2 diabetes, thus minimally processed and largely-intact wholegrain foods will likely have long-term health benefits.

- Self-reported appetite by VAS showed good agreement between controlled clinical conditions and free-living conditions. This suggest that VAS can be used to assess appetite response between diets in free-living participants.

- Overall, small differences in self-reported appetite between rye- and wheat-based meals were found. However, reduced hunger and increased fullness following the rye versus wheat-based dinner, together with lower levels of ghrelin may indicate appetite regulating properties of wholegrain rye foods after consecutive meals.

- Replacing refined wheat with wholegrain rye foods reduced glucose iAUC and measures of glycemic variability, indicating improved day-long glycemic control in individuals with overweight or obesity. While inflammatory markers GlycA and SPC were slightly elevated in the postprandial phase following rye- versus wheat-based meals, incretin responses remained largely similar across both diets.

- The hypothesis of greater reductions in body weight and fat mass with wholegrain rye versus refined wheat foods as part of a hypocaloric diet could not be confirmed in the present study. This contrasted with results from the RyeWeigth1 study and may be due to younger participants with better insulin sensitivity in the wheat-group, achieving greater weight loss in the present study.

- Wholegrain rye versus refined wheat foods caused distinct differences in gut microbiota composition and SCFA concentrations, replicating findings from the RyeWeight1 study. The rye-group showed reduced genera and species linked to negative health outcomes, along with increased plasma acetate and butyrate, potentially benefiting cardiometabolic health. Additionally, reductions in CRP suggests that wholegrain rye cereals may have beneficial effects on subclinical inflammation compared to refined wheat.

- Contrary to our hypothesis, neither appetite nor gut microbiota were linked to weight loss or fat mass reduction. Instead, metabolic factors, particularly baseline HOMA-IR and CRP, were associated with weight and fat mass loss, suggesting that individuals with elevated inflammation and insulin resistance may benefit more from wholegrain rye foods.

9 FUTURE PERSPECTIVES

- Long-term effects of whole grain particle size on body weight and effects on HbA1c and Advanced glycation end products (AGEs) in individuals with impaired insulin sensitivity should be investigated.

- Findings on improved postprandial glucose iAUC following meals based on wholegrain rye versus refined wheat should be investigated in extended intervention periods and effects on HbA1c in individuals with impaired insulin sensitivity.

- Large intervention studies including lean individuals and individuals with overweight is warranted to enable stratified analyses of metabolic responses to wholegrain rye foods across BMI-groups. Incretin hormones and other gut peptides relevant in reflecting appetite should be assessed in this context in a cross-over design.

- Associations of appetite and glycemic responses should be evaluated in lean individuals and individuals with overweight or obesity separately as altered appetite regulation have been linked with overweight.

- Measurements of SCFAs in the postprandial phase would give valuable insights to observed effects on fullness, hunger and ghrelin following the rye-based dinner.

- Further studies investigating effects of wholegrain rye and refined wheat diets on body weight should consider markers of metabolic status, including inflammation and HOMA-IR as these factors seem to influence weight loss in this context. Given the differing baseline characteristics of participants in the two RyeWeight studies, a pooled analysis could offer valuable insights into the overall impact of these diets on body weight control. Additional stratified analyses for BMI-categories might identify groups that show greater responsiveness to such dietary interventions.

- The health effects of replacing refined grains with whole grain alternatives should be evaluated against other substitution strategies within food groups. Additionally, the implementation of effective strategies must be evaluated across societal groups, accounting for variations in educational level, socioeconomic status, and acceptance.

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